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(54) Title: USE OF UREA VARIANTS AS AFFINITY LIGANDS

(57) Abstract: The present invention relates to an IgG-binding compound, which more specifically has affinity for human IgGs of k-type and functional derivatives thereof. More specifically, the compound according to the invention comprises an N,N-alkylated urea moiety located between an aromatic part and another part, which is a linear or cyclic substituted or unsubstituted aliphatic group. The compound binds to a pocket-shaped binding site present on all human IgG k-Fabs, which site is located between the two domains (CH1 and CL) of its constant part. Accordingly, the compound according to the invention is a ligand for human IgGs of k-type, and consequently, the invention also relates to a separation matrix for affinity chromatography, which matrix comprises said compound, as well as to other uses of the compound.

NEW AFFINITY LIGAND

Technical field

The present invention relates to a novel IgG-binding compound useful as a ligand for human IgGs of κ-type and functional derivatives thereof. The invention also relates to a separation matrix for use in affinity chromatography comprising said compound and various uses thereof.

Background

Antibodies, also denoted immunoglobulins, are normally synthesised by lymphoid cells derived from B-lymphocytes of bone marrow. Lymphocytes derived from the same clone produce immunoglobulin of a single amino acid sequence. Lymphocytes cannot be directly cultured over long periods of time to produce substantial amounts of their specific antibody. However, a process of somatic cell fusion, specifically between a lymphocyte and a myeloma cell, has been shown to yield hybrid cells that grow in culture and produce a specific antibody known as a monoclonal antibody. The resulting hybrid cell is known as a hybridoma. A monoclonal antibody belongs to a group of antibodies whose population is substantially homogeneous, i.e. the individual molecules of the antibody population are identical except for naturally occurring mutations.

The development of monoclonal antibody technology has provided an enormous opportunity for science and medicine in implementing research, diagnosis and therapy. Monoclonal antibodies are e.g. used in radioimmunoassays, enzyme-linked immunosorbent assays, immunocytopathology, and flow cytometry for *in vitro* diagnosis, and *in vivo* for diagnosis and immunotherapy of human disease.

Antibodies are grouped into five different types, namely immunoglobulin G (IgG), which is the most prevalent; immunoglobulin A (IgA); immunoglobulin M (IgM); immunoglobulin D (IgD); and immunoglobulin E (IgE). At present,

about thirty percent of the biotechnology-derived drugs under development are based on monoclonal antibodies of type G.

The Y-shaped disposition of the structure of the IgG molecule is well known from standard biochemistry textbooks. In brief, regarding its tertiary structure, one intact IgG molecule consists of six globular regions, each of which is formed by two domains. Regarding its primary structure, an IgG consists of two light chains and two heavy chains, which are covalently linked by disulphide bridges. The two globular parts that correspond to the "base of the Y" form the Fc fragment and are formed by domains consisting of only heavy chain residues. Contrary to this, each of the "arms of the Y" constitutes a Fab fragment with two globular parts each. Each of the globular parts in a Fab fragment is formed when one domain from the light chain contacts one domain from the heavy chain. It is well known that the globular part located further away from the centre of the antibody comprises the regions known as the hypervariable regions as well as the antigen-binding site.

By sequence homology, heavy chains of IgGs can be classified into the four types 1, 2, 3 and 4 whereas light chains fall into two types called λ and κ. In humans, about 40% of the IgG molecules carry a light chain of λ type whereas about 60% carry a light chain of κ type. IgGs built up of both light and heavy chains inherit both types of partitionings. Accordingly, one partitioning divides IgGs into four subclasses IgG1, IgG2, IgG3 and IgG4 as compared to the second partitioning which divides IgGs into two subtypes λ and κ. The same type of classification can be applied to antibody fragments like Fab fragments and so called $F(ab')_2$ fragments, which consist of two Fab fragments connected by a disulphide.

These days, IgGs are generated according to standard techniques in large quantities in cellular expression systems. The most widely used production method includes purification via chromatography, which due to its versatility and sen-

sitivity to the compounds often is the preferred purification method in the context of biomolecules. The term chromatography embraces a family of closely related separation methods, which are all based on the principle that two mutually immiscible phases are brought into contact. More specifically, the target compound is introduced into a mobile phase, which is contacted with a stationary phase. The target compound will then undergo a series of interactions between the stationary and mobile phases as it is being carried through the system by the mobile phase. The interactions exploit differences in the physical or chemical properties of the components in the sample. The interactions can be based on one or more different principles, such as charge, hydrophobicity, affinity etc. In the context of antibodies, affinity chromatography is the most widely utilised purification scheme. More specifically, affinity chromatography is a highly specific mode of chromatography wherein molecular recognition process takes place between a biospecific ligand and a target substance by a principle of lock-key recognition, which is similar to the enzyme binding to a receptor. For a general review of the principles of affinity chromatography, see e.g. Wilchek, M., and Chaiken, I. 2000. An overview of affinity chromatography. *Methods Mol. Biol.* **147:** 1-6.

Lawrence et al (J.F. Lawrence, C. Ménard, M-C Hennion, V. Pichon, F. Le Goffic, N. Durand in Journal of Chromatography A, 732 (1996) 277-281: Use of immunoaffinity chromatography as a simplified cleanup technique for the liquid chromatographic determination of phenylurea herbicides in plant material) describes an evaluation of polyclonal antibodies for cleanup of extracts of food samples. More specifically, antibodies were generated in rabbit after inoculations with an antigen prepared from an urea herbicide. Thus, the antibodies were highly specific to the urea herbicide, which is consequently not useful in any method of general antibody purification.

Another application of urea compounds is provided in EP 0 743 067 (Toray Industries), wherein the compounds are presented as highly selective adsorbing

materials used for elimination or detoxification of superantigens from body fluids. The superantigens described are enterotoxins and exotoxins, which are large proteins.

In the field of affinity chromatography, various patents and patent applications relate to protein A, which is an IgG-binding cell wall protein of the bacteria *Staphylococcus aureus,* and its use as a ligand. For example, PCT/SE83/00297 (Pharmacia Biotech AB) discloses a recombinant form of protein A, wherein a cysteine residue has been added to the protein A molecule to improve its coupling to a separation matrix for subsequent use as an affinity ligand. Further, US patent no. 6,197,927 (assigned to Genentech Inc.) discloses Z domain variants of *Staphylococcal* protein A exhibiting an IgG-binding capacity equivalent to the wild type Z domain, but a significantly reduced size. However, the binding properties of protein A are not ideal. As is well known, protein A binds to IgG molecules from various mammals, with the highest affinity to the human subclasses of IgG1, IgG2 and IgG4. It binds primarily to a surface formed at the juncture of both the second and the third constant domains, known as CH2 and CH3, of IgG located on the Fc fragment. Consequently, protein A cannot be used in affinity purification of any other fragments of IgG than Fc-containing fragments. In addition, even though protein A binds to some Fab fragments, this binding is not generic, since it targets the variable region. However, the interest in Fab and $F(ab')_2$ fragments has increased lately, since they are smaller than intact IgG molecules but still contain the functional antigen-binding region. Accordingly, the above-mentioned lack of generality becomes another drawback with protein A ligands. Moreover, in attempts to purify IgGs of subclass 3 with protein A-ligands, problems have been reported due to a precipitation of the IgG3 which precipitation is irreversible, thereby causing a loss of purified antibody. Furthermore, protein A exhibits some further drawbacks related to its being a protein. Like most proteins, it is amenable to proteolytic degradation, which may pose serious problems e.g. if a cell lysate is directly applied to a column comprising protein A-based ligand, since most cell lysates

will also comprise various proteases. Further, protein A-based ligands are usually labile to the conventionally used cleaning in place (cip) procedures at high pH conditions, which renders reuse of the column more difficult. In addition, protein A-based affinity ligands have also been known to be unstable under acidic conditions, which may result in an undesired leakage of the ligand during the purification process which will both contaminate the product and impair the quality of the purification system.

Another ligand suggested for use in affinity chromatography has been disclosed in US patent no. 4,977,247, namely the cell wall protein known as protein G. More specifically, protein G exhibits a different affinity to IgGs as compared to protein A. Protein G binds to a highly conserved region of the constant part of the Fab fragment, primarily to residues from the heavy chain, and consequently it has potential to be used as a generic Fab binder. However, it has been reported that protein G has a reduced binding to Fab fragments of type IgG2. In addition, protein G shares most of the disadvantages of protein-based affinity ligands discussed above in relation to protein A. Furthermore, many of the known protein-based affinity ligands have proven to be relatively expensive to produce.

Consequently, there is a need of novel IgG-binding ligands of a more advantageous nature, which are also more cost-effective to produce. Such new ligands should avoid the above-discussed drawbacks, and preferably also involve more preferable binding properties than the hitherto suggested ligands.

In a recent work by the present inventors, which at the time of filing of the present patent application was still not published, a novel binding site that exhibits the spatial conformation of a pocket was identified. The binding pocket was shown to be specific for human kappa IgGs of all subtypes.

The recently identified binding pocket directed the present inventors to a new target on the human IgG molecule in their efforts to find a new affinity ligand with improved properties as compared to the prior art.

Summary of the present invention

One object of the present invention is to provide a novel ligand to human IgG-molecules of κ-type, which avoids one or more of the above-discussed disadvantages.

A specific object of the present invention is to provide a novel ligand to human IgG-molecules of κ-type, which is general for all subclasses of said IgGs.

Another object of the invention is to provide a novel ligand to human IgG-molecules of κ-type, which is capable of specific binding to said IgGs.

Yet another object of the present invention is to provide a novel ligand to human IgG-molecules of κ-type, which conforms spatially with a binding pocket defined by the amino acids of the interacting surfaces defined in Fig 2, or with essential parts thereof.

An additional object of the present invention is to provide a novel ligand to human IgG-molecules of κ-type, which exhibits more advantageous chemical properties than protein-based affinity ligands e.g. at extreme pH values and which is more cost-effective to produce.

Further objects and advantages of the present invention will appear from the detailed description that follow below.

Brief description of the drawings

Figure 1 shows the executed synthetic route to variations of the substitution pattern of a compound according to the invention and also outlines how in the experimental part below, the compounds in the directed library were provided with a handle for immobilisation.

Figure 2 shows orthographic views of the herein-discussed binding pocket in chicken net model.

Figure 3 shows a selection of compounds according to the invention, wherein the substitution pattern has been varied.

Figure 4 shows orthographic views of some of the compounds derived from AB_0001250.

Figure 5 A-E show orthographic views of the docked compounds AB_000125[1-5].

Figure 6A and B show the structure coordinates of the amino acids that form the interacting surfaces of a binding pocket, which is specific for human IgGs of κ-type. Said binding pocket, and compounds comprising said interacting surfaces, were identified by the present inventors and claimed in a separate patent application, which was still pending, but not public at the time of the present filing.

Figure 7 shows the results of affinity chromatography on a separation matrix according to the invention, wherein a Fab-fragment of κ-type is successfully isolated.

Figure 8 shows the results of affinity chromatography on a separation matrix according to the invention, wherein another Fab-fragment of κ-type is successfully isolated.

Figure 9 shows as a comparative test an attempt to isolate a Fab-fragment of lambda-type by affinity chromatography on a separation matrix according to the invention.

Definitions

The terms "antibody of κ type", "Fab fragment of κ type" and "$F(ab')_2$ fragment of κ type" mean herein an antibody, a Fab fragment and an $F(ab')_2$ fragment respectively, wherein the light chain is of κ type.

The term "ligand" means herein a chemical entity capable of specific binding to a target.

The term "associating with" refers to a condition of proximity between a chemical entity, or portions thereof, and a target i.e. a binding pocket or binding site on a protein. The association may be non-covalent, wherein the juxta-

position is energetically favoured by hydrogen bonding or van der Waals or electrostatic interactions, or alternatively it may be covalent.

The term "functional derivative" is used to mean a chemical substance that is related structurally and functionally to another substance. Thus, a functional derivative comprises a modified structure from the other substance, and maintains the function of the other substance, which in this instance means that it maintains the ability to interact with the same ligands. Thus, a "functional derivative" can be either a natural variation or fragment thereof, or a recombinantly produced entity. In addition, a "functional derivative" can also comprise added molecules or parts, as long as the described function is essentially retained.

The term "binding pocket", as used herein, refers to a region of a molecule or molecular complex, that, as a result of its hollow shape, favourably contributes to the molecule's association with another chemical entity.

The term "interacting surface" means herein a surface comprised of residues capable of interacting with a binding molecule or other entity, e.g. by ionic attraction, hydrogen bonds, Van der Waals interaction etc.

The term "strictly conserved" is used herein to mean that after a sequence alignment of all sequences available from an internationally recognised sequence database, the residue type is exactly the same at a specific position for all aligned sequences. An example of such a database is the non-redundant database provided by the National Centre for Biotechnology Information.

The term "structure coordinates" refers to Cartesian coordinates derived from for example mathematical equations related to the patterns obtained on diffraction of a monochromatic beam of X-rays by the atoms (scattering centres) of a protein or protein-ligand complex in crystal form. The diffraction data are used to calculate an electron density map of the repeating unit of the crystal. The electron density maps are then used to establish the positions of the individual atoms of the protein or protein complex.

A "pharmacophore" is defined herein as the assembled atoms or centres in a target molecule, which have critical interactions with a receptor. Some types

commonly used include hydrogen bond donors; hydrogen bond acceptors; positively or negatively charged centres; aromatic ring centres; and hydrophobic centres.

The term "docking" means herein a fitting operation, wherein the ability of a chemical entity to bind or "dock" to a binding site is evaluated.

The term "library" means a collection of molecules or other chemical entities with different chemical structures and/or properties.

The term a "Conolly surface" defines the surface of the volume accessible to a hard spherical probe of a given radius, usually taken as 1.4Å, which is the radius of water in ice form. This surface can be obtained by "rolling the probe" over the atoms of the protein.

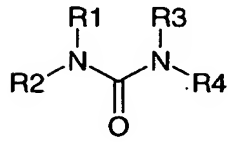
Detailed description of the invention

A first aspect of the present invention is a compound capable of associating with human IgGs of κ-type and functional derivatives thereof. More specifically, the present compound is capable of specific and reversible binding to a binding pocket of a human IgG of κ-type, which binding pocket is defined by the structure coordinates for the amino acids that constitute the interacting surfaces as shown in Fig 6. Said binding pocket was recently identified by the present inventors, and is located between the two domains (CH1 and CL) of the constant part of κ-Fab. Thus, the herein discussed binding pocket provides a novel binding site for human IgGs of κ-type, which binding site is a general binding site for all such IgGs as well as fragments or functional derivatives thereof.

The present invention is based on an evaluation of a large number of potential binders to κ-Fab of human IgGs, wherein virtual screening hits were tested with NMR. The results from the NMR was subsequently utilised to derive structure-activity relationships that led to the construction of a pharmacophore, and a library of affinity ligands was then designed to optimise binding and include a handle for immobilisation to a chromatographic support. As will be

disclosed in detail in the Experimental part below, the present inventors have studied different substitution patterns and evaluated a wide range of structures in order to identify the features required for a compound to exhibit a satisfactory binding to human IgGs of κ-type via the above discussed binding pocket.

More specifically, the compound according to the invention is based on an N,N-alkylated urea moiety located between an aromatic part and an aliphatic part. In the most preferred embodiment, the present invention is an IgG-binding compound represented by formula (I) below



(I)

wherein

R_1 is CH_3 or CH_2CH_3;

R_2 is a *para* and/or *meta* substituted phenyl group;

R_3 is H, CH_3 or CH_2CH_3; and

R_4 is a linear or cyclic aliphatic group, which is optionally substituted,

or, wherein

R_1 and R_2 are as stated above while R_3 and R_4 are both parts the same 4- to 6-membered cyclic entity, which is optionally substituted,

and which compound has affinity for human IgG of κ-type.

Thus, in one embodiment, the compound is an affinity ligand with affinity for a Fab fragment of human IgG of κ-type. In some contexts, such an affinity ligand is denoted an affinity adsorbent or an antibody adsorbent.

As the skilled person in this field will easily appreciate, in formula (I), the bonds between the carbonyl carbon and each one of the nitrogen atoms are rotatable. Consequently, position R_1 is equivalent to position R_2 and position R_3

is equivalent to position R_4, and the definitions herein will encompass any definition of a compound, wherein R_1 has been interchanged with R_2 as well as when R_3 has been interchanged with R_4. Likewise, because of the inherent symmetry around the keto group, the pair R_1/R_2 is interchangeable with the pair R_3/R_4 so all these definitions are also included.

In an advantageous embodiment of the compound, in formula (I), R_1 is CH_3.

As mentioned above, in formula (I), R_2 is a phenyl group, which may be substituted with one or two halogens, such as F, Cl, Br, or I. Since substituents in *ortho* position have been observed to have a negative impact on binding, any substituents are present in *meta* and/or *para* position. Thus, in a specific embodiment, R_2 is substituted with Cl or F in the *meta* position. In another embodiment, R_2 is substituted with Cl in the *meta* position and F in the *para* position. In another embodiment, R_2 is substituted with F in the *meta* position and Cl in the *para* position. In yet another embodiment, R_2 is substituted with Cl in *meta* and *para* position.

Alternatively, or additionally, the R_2 phenyl group is substituted with one or more oxygen-comprising groups. Thus, in one embodiment, R_2 is a substituted phenyl group and the substituents are selected from the group that consists of F, Cl, Br, I and OH, preferably F and Cl.

In a specific embodiment, R_2 is substituted in the *para* and/or *meta* position with a group defined as $-O-R_5$, wherein R_5 is CH_3 or CH_2CH_3, and preferably CH_3.

As appears from the modelling described in the experimental part below, when the present compound binds to an IgG molecule, R_2 will be located in the inner part of the pocket and hence interact with the inner amino acids of the interacting surfaces of the binding pocket. Larger ring-systems than six-membered

rings were according to NMR screening described in the experimental part below found to have a negative influence on binding, and are hence avoided. Also, as mentioned above, in the most preferred embodiment, the aromatic group does not comprise any heteroatoms, since especially the presence of nitrogen atom(s) in the ring has been observed to have a negative impact on binding. However, in an alternative embodiment, the invention is a compound represented by the chemical formula (I) as defined above, wherein R_2 is another aromatic group than phenyl. In the most preferred embodiment of this alternative, R_2 comprises thiophene.

As mentioned above, R_3 can be H or CH_3 or CH_2CH_3.

As mentioned above, in formula (I), R_4 can be a linear or cyclic aliphatic group, which is substituted or unsubstituted. In this context, an aliphatic group can be any linear or branched carbon chain interrupted by any heteroatom, as long as the compound fits sufficiently well in the herein-defined binding pocket to provide binding thereof. In one embodiment, the aliphatic chain comprises one or more carbonyl group(s).

In one alternative embodiment, R_4 is an aromatic group that comprises a phenyl group. In one embodiment, said phenyl group is substituted in the *ortho* and/or *meta* and/or *para* position. In a specific embodiment, said phenyl group comprises one or more heteroatoms, such as N, S etc.

In a specific embodiment, R_4 can be a methyl-substituted amino acid residue, or a derivative thereof. Thus, in a specific embodiment, R_4 is selected from the group that consists of aliphatic amino acid residues, hydroxyl-containing amino acid residues, sulphur-containing amino acid residues, aromatic amino acid residues, acidic amino acid residues, basic amino acid residues or imino-containing amino acid residues, or any derivative thereof.

In a specific embodiment, which is especially advantageous if the compound is to be used in a form immobilised to a solid support, e.g. as a ligand in affinity chromatography, the aliphatic group R_4 also comprises terminating functionalities useful for such immobilisation. Thus, in one embodiment, an aliphatic group is a linear or branched carbon chain as discussed above, which is terminated with a carboxylic acid i.e. -COOH. In an alternative embodiment, the aliphatic group is terminated with a carboxylic acid derivative, such as an ester, a halide, an amide, a nitrile or the like. In an alternative embodiment, an aliphatic group is a linear or branched carbon chain as discussed above, which is terminated with nitrogen, oxygen, sulphur or any derivative thereof. Such derivatives are well-known to the skilled person in this field, and are also useful for immobilisation. As mentioned above, the only limitation in this context is that the aliphatic group does not impair the binding of the compound to the herein defined binding pocket.

In another embodiment, in formula (I), R_4 is CH_3. In a specific embodiment, both R_3 and R_4 are CH_3. In a specific embodiment, in formula (I), R_1 is CH_3; R_2 is a phenyl group that has been substituted with Cl in *meta* and *para* position; R_3 is CH_3; and R_4 is CH_3. In one embodiment, the present compound is selected from the compounds shown in Figure 3.

As also appears from the above, in an alternative embodiment, R_3 and R_4 are parts of a 4- to 6-membered cyclic entity. In an advantageous embodiment, the cyclic entity is 3- to 5-membered. Consequently, said cyclic entity comprises the N of Formula (I), R_3 and R_4 and optionally 1 or 2 other atoms, which may be carbon atoms or heteroatoms. In the most preferred embodiment, the R_3 and R_4 substituents constitute an amino acid derivative. In one embodiment, R_3 and R_4 are part of a 5-membered cyclic entity, which in turn is substituted, preferably with a group useful for immobilisation as discussed above. In a specific embodiment, a 5-membered cyclic entity is substituted in the position adjacent to the N with a C(O)-O-CH3 group, and consequently the R_3 and R_4 substitu-

ents of this embodiment are parts of a D-proline derivative. This specific embodiment is denoted AB_0003290 in Figure 3.

Furthermore, the present invention also encompasses a compound, which is basically represented by formula (I) above, but wherein R_1 and R_3 are carbon atoms connected to each other to form a cyclic structure. In this embodiment, R_3 is a carbonyl group. In this embodiment, R_4 is preferably a phenyl group. Thus, this embodiment of the compound is known as 1,3-diphenylimidazolidine-2,4-dione.

In order to provide the best binding to the herein-discussed binding pocket of a human IgG of κ-type, or to a functional derivative thereof, it is preferable that the compound has a non-planar geometry. In the context of the binding pocket, it is noted that the present compound is capable of binding to binding pockets not only of the exact defined structure coordinates as defined herein, but also to pockets defined by interacting surfaces having a mean square deviation from the backbone atoms of the disclosed binding pocket amino acids of not more than 2.0Å . In a preferred embodiment, said deviation is not more than about 1.5Å and in the most preferred embodiment, said deviation is not more than 1.0Å. In one embodiment, the present compound is capable of binding to a human IgG or a functional derivative thereof with a binding constant of at least 10^{-3} M, preferably at least 10^{-6} M and most preferably at least 10^{-8} M. Thus, illustrative intervals of such binding are e.g. 10^{-3} M^{-4} to 10^{-8} M, such as 10^{-3} M^{-4} to 10^{-6} M or 10^{-6} to 10^{-8} M.

In a specific embodiment, the present compound is capable of binding to a human IgG of κ-type, or a functional derivative thereof, via a binding pocket formed between two polypeptides, wherein the first polypeptide is the portion of a human IgG κ light chain that starts at one of amino acids 93 to 110 and ends at one of amino acids 187 to 214 of human IgG κ light chain and the second polypeptide is the portion of a human IgG heavy chain that starts at one of

amino acids 106 to 128 and ends at one of amino acids 215 to 225 of human IgG heavy chain. In the herein used enumeration of amino acids refers to a human sequence wherein no. 93 is the first amino acid of the constant domain, as also used in Figure 6.

Thus, the IgG-binding compounds according to the present invention are in general smaller than the prior art affinity ligands used for antibody isolation. In addition, the compounds according to the invention are organic molecules that lack the peptide structure of e.g. protein A- and protein G-based ligands, which in general renders them less susceptible to extreme pH values. Naturally, they are not as susceptible to proteolytic degradation, or any other kind of degradation, as the protein-based prior art ligands either. In addition, the present compounds are more cost-effective to produce.

The compound according to the invention can be prepared by the skilled person in this field using well-known methods, as illustrated e.g. in Figure 1 below and as explained in the experimental part below under "Synthesis".

A second aspect of the invention is the use of a compound as defined above for selective binding of human IgG of κ-type, or a functional derivative thereof. In the present context, it is understood that the encompassed derivatives can be any human κ-Fab constant part- comprising compounds, i.e. any composition comprising the globular region of an IgG molecule formed by the first constant domain of the heavy chain (CH1) and the constant domain of the light chain (CL). Thus the term includes any of the following terms which are well known from standard IgG terminology: Intact IgG molecules, $F(ab')_2$ fragments, Fab' fragments, Fab fragments and by definition the globular region named itself, all of which have human sequences and light chains of κ-type. This definition includes also any modifications of named IgG or named antibody fragments including even chimeric molecules formed in one part of one of said compositions and in another part of any of the following proteins, peptides, carbohy-

drates, lipids or any other organic or inorganic entity and chimeric combinations thereof and also any of the above-mentioned covalently attached to solid phase.

The present invention also encompasses a sorption complex comprised of a compound as defined above directly linked to the Fab fragment of a human IgG of κ-type, or a functional derivative thereof. More specifically, the compound is linked to the Fab fragment of said antibody, and more specifically to the herein described binding pocket. Such a sorption complex will form as the compound according to the invention is contacted with a solution comprising human IgG's of κ-type, or a functional derivative thereof, under suitable conditions. The skilled person in this field can easily select such conditions and adjust pH, ionic strength etc to provide or to break up the complex.

Another aspect of the invention is a separation matrix for use in affinity chromatography, wherein the ligands comprises at least one compound as defined above. In a specific embodiment, the ligands have been coupled to a support via linkers. The present matrix can e.g. be in the form of separate particles, preferably porous and essentially spherical particles; a monolith; or a membrane.

The present invention also encompasses a system suitable for affinity chromatography, which is comprised of a separation matrix as defined above packed in a column. The column may be of a size suitable for analytical scale or for large scale chromatography.

Suitable support materials are well known. In one embodiment, the support is a natural polymer, such as agarose, alginate, carrageenan, gelatine etc. Such natural polymers are known to form physically cross-linked networks spontaneously on cooling or on addition of divalent metal ions, and chemical cross-linkers can be added if desired. This kind of supports is easily prepared ac-

cording to standard methods, such as inverse suspension gelation (S Hjertén: Biochim Biophys Acta 79(2), 393-398 (1964). In another embodiment, the support is comprised of cross-linked synthetic polymers, such as styrene or styrene derivatives, divinylbenzene, acrylamides, acrylate esters, methacrylate esters, vinyl esters, vinyl amides etc. Such polymers are also easily produced according to standard methods, see e.g. "Styrene based polymer supports developed by suspension polymerization" (R Arshady: Chimica e L'Industria 70(9), 70-75 (1988)). Thus, in summary, the support material can in principle be any material that allows the covalent coupling of the IgG-binding compound discussed above, such as the above-discussed polymers, inorganic materials, such as silica, ceramics etc.

Many well-known methods are available for immobilising ligands to a support through suitable functional groups. As the skilled person in this field will realise, the exact choice of coupling method will depend on the structure of the ligand to be immobilised. In one embodiment, the support has hydrophilic surfaces, and if porous, the surfaces of the pores are also hydrophilic. This is advantageous in order to avoid or at least reduce any non-specific protein interactions. It is also advantageous if the surfaces have a high density of groups available for coupling of ligands. Such coupling groups are commonly hydroxyl groups, but may also be allyl groups i.e. double bonds available for grafting, amines, thioles, epoxides and the like. If the support material has undesirable surface properties, it is possible to coat it with a hydrophilic polyhydroxy-functional material before coupling the ligand. The techniques and considerations for coupling of affinity ligands to a suitable support to prepare a separation matrix are well known in this field, see e.g. WO 98/33572 for a detailed review of coupling chemistry as well as suitable linking molecules, therein denoted "extenders".

Another aspect of the invention is a generic method of isolating or separating a target compound, i.e. a human IgG of κ-type, or a functional derivative thereof,

from other components in a liquid, wherein a compound or a separation matrix as defined above is used. In the context of immunology, the separation matrix is often denoted an "immunsorbent". In the most preferred embodiment, the present method is affinity chromatography, which is a widely used and well-known separation technique. In brief, in a first step, a solution comprising the desired antibodies is passed over a separation matrix under conditions allowing adsorption of the antibody to ligands present on said matrix. Such conditions are controlled e.g. by pH and/or salt concentration i.e. ionic strength in the solution. Care should be taken not to exceed the capacity of the matrix, i.e. the flow should be sufficiently slow to allow a satisfactory adsorption. In this step, other components of the solution will pass through in principle unimpeded. Optionally, the matrix is then washed, e.g. with an aqueous solution, in order to remove retained and/or loosely bound substances. In a next step, a second solution denoted an eluent is passed over the matrix under conditions that provide desorption i.e. release of the desired antibody. Such conditions are commonly provided by a change of the pH, the salt concentration i.e. ionic strength, hydrophobicity etc. Various elution schemes are known, such as gradient elution and step-wise elution. Elution can also be provided by a second solution comprising a competitive substance, which will replace the desired antibody on the matrix.

In an alternative embodiment, the compound according to the invention is used in site-specific modification of a human IgG of κ-type, or a functional derivative thereof. More specifically, a human IgG of κ-type, or a functional derivative thereof, can be modified by binding a compound as defined above selectively to the binding pocket identified by the present inventors. In a specific embodiment, the modification is a stabilisation of Fab-folding.

In an alternative embodiment, the present compound is used in an immunological assay for detection of a human IgG of κ-type, or a functional derivative thereof. In this case, the compound is preferably labelled with a suitable detect-

able label as conventionally used, such as a fluorescent label, a luminescent label, a chemiluminiscent label, an enzyme label, a radioactive label, an absorbance label etc. Such assays may be in solution or on solid phase. In one embodiment, the human κ-Fab constant part-comprising composition is a human IgG or a fragment thereof. In the preferred embodiment, the present assay is a competitive assay, wherein the ability of a candidate ligand to displace a known ligand's binding to a compound or binding pocket as defined above is evaluated.

Detailed description of the drawings

Figure 1 shows the executed synthetic route to i) variations of the substitution pattern and ii) provide the compounds in the directed library with a handle for immobilisation as discussed below in Example 2. To the top-left, the original hit AB_0001250 is shown. The synthesis will be described in detail below in the section Materials and methods.

Figure 2 shows orthographic views of the herein-discussed binding pocket in chicken net model. The amino acid residues forming the pocket are shown in stick model and the corresponding structure coordinates are presented in Figure 6. Docked hit AB_0001250 is shown in space-fill model to illustrate the possibilities of the pocket to harbour a substituted phenyl ring.

Figure 3 shows a selection of compounds according to the invention, wherein the substitution pattern of R_1, R_2 as well as R_3 and R_4 has been varied. A central N,N-alkylated urea moiety as well as a *para* and/or *meta* substituted phenyl groups are present in all the compounds.

Figure 4 shows orthographic views of compounds derived from AB_0001250. Five docked hits superimpose very well onto the original hit AB_0001250.

Figure 5 A-E show orthographic views of the docked compounds AB_000125[1-5] in the binding pocket, as discussed in more detail in the experimental part below.

Figure 6 A and B show the structure coordinates of the amino acids that form the interacting surfaces of the binding pocket shown in Figure 2, which is spe-

cific for human IgGs of κ-type. Figure 6A shows the coordinates of the light chain, while Figure 6B shows the heavy chain. More specifically, the structure coordinates shown form a small pocket in between the two domains (CH1 and CL) of the constant part of κ-Fab and constitutes a novel target binding site. The residues forming the pocket together with some residues located at the entrance and contributing significantly to the topology of the putative binding site have been identified as follows. From the light chain, there are **Q124**, **S127**, **G128**, **T129**, **S131**, **V133**, **G157**, **N158**, **S159**, **Q160**, **E161**, **S162**, **S176**, **S177**, **T178**, **T180**, **L181**, and they are all strictly conserved for all sequences of κ-type identified in a sequence homology search. The residues from the heavy chain are K126, **P128**, **S129**, F131, **L133**, **L150**, **K152**, D153, **F175**, **P176**, **V178**, **L179**, **Q180**, S181, S182, L184, S186, **L187** and **S188**, bold being strictly conserved and remaining highly conserved. The structure coordinates of the full amino acid sequence of a human IgG of κ-type can be obtained from the Protein Data Bank, accession code 1vge, e.g. at –http://www.rcsb.org/pdb/.

Figure 7 illustrates how a Fab-fragment of a monoclonal antibody of kappa-type can be isolated by affinity chromatography using a separation matrix according to the invention, as described in Example 6 below. Injection of the monoclonal ABFab-K1 on the AB_0003291-containing medium according to the invention in PBS, 1 M $(NH_4)_2SO_4$, pH 7 at 0 ml. Cleaning in place (CIP) starts at 10.0 ml. The small peak at 1.07 ml is due to injection effect. The protein is not totally removed from the column during the CIP. Evidently, the affinity column is able to bind the monoclonal ABFab-K1.

Figure 8 illustrates how another Fab-fragment of a monoclonal antibody of kappa-type can be isolated by affinity chromatography using a separation matrix according to the invention, as described in Example 6 below. Injection of the monoclonal ABFab-K2 on the AB_0003291-containing media in PBS, 1 M $(NH_4)_2SO_4$, pH 7 at 0 ml. CIP starts at 10.0 ml. The small peak at 1.09 ml is due to injection effect. The protein is not totally removed from the column during the CIP. Evidently, this affinity column is also able to bind the monoclonal ABFab-K2, and the compound according to the invention can conse-

quently be described as a ligand useful as a general binder of human IgG Fab fragments of κ-type.

Figure 9 illustrates as a comparative test how a Fab-fragment of a monoclonal antibody of lambda-type is tested in affinity chromatography on a separation matrix according to the invention, as described in Example 6 below. Injection of the monoclonal ABFab-L2 on the AB_0003291-containing media in PBS, 1 M $(NH_4)_2SO_4$, pH 7 at 0 ml. CIP starts at 10.0 ml. From this figure, it clearly appears that the ABFab-L2 directly comes off the affinity column and is found in the flow-through. Accordingly, the separation matrix according to the invention is not suitable for isolation of Fab-fragments of lambda-type, and confirms the statement above that the compound according to the invention is a binder of human IgG Fab fragments of κ-type, but not of lambda-type.

EXPERIMENTAL PART

Below, the present invention will be explained in more detail by way of examples, which however are not to be construed as limiting the present invention as defined by the appended claims. All references given below and elsewhere in the present specification are hereby included herein by reference.

Materials and Methods

Molecular Modelling

Compounds of the directed library were sketched with MDL ISIS/draw and transferred to an OCTANE™ (Silicon Graphics Inc.®) workstation provided with two 195MHz R10000 processors. The program package SYBYL® (Tripos Inc., 2000) was used for all remaining modelling.

Preparation of compounds for docking

The structures of the compounds were transformed into 3D using the program CONCORD and ionised to reflect their most probable protonation state at pH 7. The coordinates were then subject to 500 cycles of minimisation using the

MMFF94 force field (Halgren 1996 – Halgren, T. 1996. Merck molecular force field. I. Basis, form, scope, parameterisation, and performance of MMFF94. *J. Comp. Chem.* **17:** 490-519.).

Docking of prepared molecules

Docking simulations have been performed with the program FlexX™ (Rarey et al. 1996 Rarey, M., Kramer, B., Lengauer, T., and Klebe G. 1996. A fast flexible docking method using an incremental construction algorithm. *J. Mol. Biol.* **261:** 470-489.) which is part of the SYBYL package. FlexX allows flexibility in the ligands, keeping the receptor fixed. All the relevant receptor information necessary for the docking simulations is stored in the receptor definition file (rd file). FlexX uses formal charges, which were turned on during the docking simulations. The protein structure used was the highest-resolution (2.0 Å) crystal structure of κ-Fab (accession code to the Protein Data Bank 1vge, Chacko *et al.,* 1996 Chacko, S., Padlan, E. A., Portolano, S., McLachlan, S. M., Rapoport, B.: Structural studies of human autoantibodies. Crystal structure of a thyroid peroxidase autoantibody Fab. *J Biol Chem* 271 *pp.* 12191 (1996)). The following residues were included in the definition of the binding site: from the light chain: Ser-131, Val-133, Ser-159, Gln-160, Glu-161, Ser-162, Ser-176, Thr-178, and Thr-180. From the heavy chain: Leu-150, Lys-152, Phe-175, Pro-176, Val-178, Gln-180, Ser-186, Leu-187, Ser-188. All of these residues have previously been shown by the present inventors to be strictly conserved as observed from a sequence alignment and are a subset of the identified pocket. The subset was created by taking all residues with at least one atom at a distance of at least 4 Å from the docked hit AB_0001250 and subsequently by including some additional residues to complete a Conolly surface of the pocket surrounding the docked hit. In the protein structure, the ε carbonyl oxygen of H:Gln-180 is located 2.5Å away from one of the δ carboxyl oxygens of H:Asp153. This was assumed to be an error due to misinterpretation of the electron density of the carboxyamide terminal group of H:Gln-180, and the group was consequently flipped around 180°. In this corrected structure, the ε

nitrogen of Gln-180 from the heavy chain is at favourable hydrogen bonding distance to the carboxyl oxygen of H:Asp153. Otherwise, defaults have been used when creating the rd file and no special customisations have been done. When necessary the *SYBYL LINE NOTATION (sln) core* option of FlexX in SYBYL was applied to bias the docking towards conformations that were compatible with the expected binding mode with the phenyl ring inside the pocket. The *sln core* option was applied with input *N(C(NCH3)=O)(C[9]:CH:CH:C:C:CH:@9)CH3* to indicate to the program to start fragment build-up using a common substructure of the six compounds in the directed library. Prior to docking, all water molecules were removed. The 30 best ranked conformations and their FlexX score were saved for each molecule.

Synthesis of library based on AB_0001250

Synthesis of 4-(methylamino) butyric acid methyl ester

4-(methylamino) butyric acid·HCl was dissolved in methanol and thionyl chloride in catalytic amount was added drop by drop. The reaction mixture was stirred at 0 °C for 30 min. Thereafter, the solvent was reduced *in vacu*, yielding a white solid.

Synthesis of 3,4-dichloro-/(N-methyl)-aniline

3,4-dichloro aniline (40 mmol, 5 g) was dissolved in 400 mL of DCM. To this solution was added iodo methane (40 mL), triethyl amine (5 mL), and NaH (40 mmol, 3.8 g). The resulting mixture was stirred at ambient temperature over night, where after small aliquots of water summing up to a total of 50 mL of water was added, followed of an additional hour of stirring. The reaction mixture was transferred to a separation funnel and extracted with 5 % sodium thiosulphate, dried over magnesium sulphate and concentrated *in vacu* to almost complete dryness. The material was separated by silica chromatography (pentane:ether – 8:2), the appropriate fractions were collected and concentrated *in vacu* to almost complete dryness, yielding 3g of material including some sol-

vent. The correct material was indicated by LC-MS analysis. This material was directly used in the subsequent step.

General method for synthesis of N-methylated aniline derivatives

The aniline derivative was dissolved in DCM and sodium hydride (in the case of AB_0001253 sodium bis(trimethylsilyl) amide) (1.5 eq) and di-tertbutyl-di-carbonate (1.3 eq) was added followed by stirring at room temperature over night. The reaction mixture was transferred to a separatory funnel and extracted with water, dried over magnesium sulphate, and concentrated *in vacu*. The crude product was dissolved in THF and lithium alumina hydride (1.2 – 2 eq) was added and the reaction mixture was refluxed until completion as indicated by LC-MS. Thereafter the mixture was filtered. This filtrate was used directly in the subsequent step.

General method for synthesis of urea derivatives

To a THF solution of the N-methylated aniline (or the non-N-methylated aniline derivative) was added phosgene (20% in toluene) in large excess and the reaction mixture was stirred at room temperature for 30 min, concentrated *in vacu*, and re-dissolved in DCM. To this solution was added an excess of triethyl amine and 4-(methylamino) butyric acid methyl ester (or 4-amino butyric acid methyl ester) (approx. 1 eq). The reaction mixture was stirred at room temperature for 3 hours, concentrated *in vacu*, and purified by RP-HPLC.

General method for hydrolysis of methylesters

The methyl ester of the urea derivative (0.5 g) was dissolved in methanol (10 mL) and lithium hydroxide (0.25 g) was added. The resulting mixture was stirred at ambient temperature for 5 hours, neutralised with 1 M HCl, and concentrated *in vacu*. The resulting material was purified by RP-HPLC.

Synthesis of 1-(3,4-dichlorophenyl)-1,3-dimethyl-3-butyric acid urea

3,4-dichloro-N-methyl-aniline (all material from previous description) was dissolved in 200 mL of DCM. To the solution was added phosgene (20 mL, 20%

sol. in toluene) and the mixture was stirred for 30 minutes at ambient temperature. The solvent was removed *in vacu* and an additional 100 mL of DCM was added, followed by removal of the added solvent *in vacu*.

The remaining solid was dissolved in 200 mL of DCM and 4-methyl-4-amino butyric acid (2 g) was added followed by the addition of triethyl amine (5 mL). The resulting mixture was stirred at ambient temperature during 2 hours. Thereafter, the reaction mixture was transferred to a separation funnel and partitioned between DCM and water. The organic phase was isolated, dried over magnesium sulphate, and concentrated *in vacu*. The remaining material was purified by silica column chromatography (DCM:Et-OH – 9:1), the appropriate fractions were collected and concentrated *in vacu* to yield 1.4 g of the desired material as a clear oil.

Example 1: Binding test using NMR

All NMR experiments were performed at 298 K on a Bruker Avance 500 MHz spectrometer. The 1D saturation transfer difference method (STD-NMR) was used as screening assay (Mayer M. and Meyer B. 1999. Characterisation of Ligand Binding by Saturation Transfer Difference NMR Spectroscopy. *Angew. Chem., Int. Ed.* **38:** 1784-1788). The resulting STD-NMR spectrum shows the difference between spectra recorded with on- and off-resonance irradiation of the protein, respectively. The two spectra are recorded in the same experiment in an interleaved fashion. If the resulting STD-NMR spectrum shows the same signals as the reference ^{1}H-NMR spectrum of the ligand the result is regarded as positive *i.e.* the ligand must have contacted the protein. Ligands that do not have any contact with the protein or are very tightly bound to the protein will not give any signal in the resulting STD-NMR spectrum. It has been shown that the method is capable of detecting ligands with dissociation constants between 10^{-3} and 10^{-8} M (Mayer M. and Meyer B. 1999. Characterisation of Ligand Binding by Saturation Transfer Difference NMR Spectroscopy. *Angew. Chem., Int. Ed.* **38:** 1784-1788). The strength of the STD-NMR signal depends upon

several factors including protein size, offset and duration of the on-resonance irradiation, the dissociation rate constant and the excess of ligand. The STD-NMR method is advantageous in that the detection limits can be tuned for binding by varying the protein concentration while keeping the ligand concentration constant. Under such conditions, at higher protein concentrations the weak to medium binders are detected, whereas at lower protein concentrations only medium binders are detected. For instance, it has been shown before for another enzyme system that both μM and mM binders were detected at a protein concentration of 35 μM whereas only μM binders were detected at protein concentrations of 1 μM and 100 nM (Peng J. W., Lepre C. A., Fejzo J., Abdul-Manan N. and Moore J. M. 2001, Nuclear Magnetic Resonance-Based Approaches for Lead Generation in Drug Discovery, Methods in Enzymology. **338:** 202-230). It should be noted that the signal intensity at one specific protein concentration should not be taken as a direct measure of the binding strength. For instance, in the same study, a mM binder showed a stronger signal as compared to a μM binder at 35 μM protein concentration whereas when the protein concentration was reduced to 1 μM the signal from the weaker binder vanished. On the other hand the signal of the μM binder became even stronger than before (Peng J. W., Lepre C. A., Fejzo J., Abdul-Manan N. and Moore J. M. 2001, Nuclear Magnetic Resonance-Based Approaches for Lead Generation in Drug Discovery, Methods in Enzymology. **338:** 202-230).

Here, three different antibody concentrations were used, namely, 0.5 μM, 100 nM and 20 nM. The antibody used was a human Fab of κ–type. In all cases ligands were tested one-by-one. On-resonance irradiation was set at 0 ppm and off-resonance irradiation was set at –40 ppm. Irradiation time in each scan was 2 s and 16K data points were collected with 1024 scans in total. Compounds for testing were dissolved in $DMSO_{d6}$ to a concentration of 50 mM and 5 μL of the concentrated ligand solution was added to 495 μL buffer solution. The samples thus consisted of 0.5 mM ligand, 20 mM phosphate buffer, 100 mM NaCl and 5% $DMSO_{d6}$ in D_2O at pD 7.5, uncorrected reading on pH-meter.

Compounds were initially tested for binding with 0.5 μM antibody. Interesting ligands were further tested with protein concentrations of 100 or 20 nM. A one-dimensional ^{1}H-spectrum was acquired first as reference spectrum and subsequently a saturation transfer difference (STD) spectrum was acquired. Each analysis took 60 minutes on the spectrometer.

The results are shown in Table 1 below, wherein the results from NMR screening are compiled.

Table 1: Results from the NMR screening

Concentration code as follows: conc. 1 means 500, conc 2 100 and conc 3 20 nM protein. NMR signal code: 0 no, 1 weak and 2 strong signal.

ID	Chemical name	conc 1	conc 2	conc 3	ctrl
AB_0000510	alpha-pyridoin	0			
AB_0000530	1-(3-chlorophenyl)-3-methyl-2-pyrazolin-5-one	0			
AB_0000540	1,3-diphenylparabanic acid	0			
AB_0000580	n1-(3-chloro-4-fluorophenyl)-2-[(4,6-dimethylpyrimidin-2-yl)thio]acetamide	1	0		
AB_0000600	3-phenyl-1,2,4-benzotriazine	1	1		0
AB_0000610	2-(4-chlorophenyl)-2,3-dihydro-1h-pyrrolo[3,4-c]pyridine-1,3-dione	0			
AB_0000630	n1-(2,3,4-trifluorophenyl)-2-(1,2,4-oxadiazol-3-yl)acetamide	0			
AB_0000670	methyl n-[(5-methyl-4-phenyl-1,3-oxazol-2-yl)carbonyl]carbamate	2	0		
AB_0000690	n1-(2,4-difluorophenyl)-2-(1,2,4-oxadiazol-3-yl)acetamide	0			
AB_0000700	3,6-di-2-pyridyl-1,2,4,5-tetrazine	0			
AB_0000730	2-benzylidene-1,3-indandione	0			
AB_0000740	5-phenyl-1,2,4-oxadiazol-3-yl n-(4-fluorophenyl)carbamate	0			
AB_0000750	n-(5,5-dimethyl-7-oxo-4,5,6,7-tetrahydro-benzothiazol-2-yl)-nicotinamide	0			
AB_0000760	5-(2-phenyl-1,3-thiazol-4-yl)-	1	0		

	1,3,4-oxadiazol-2-ylhydrosulfide				
AB_0000790	3-[2-oxo-2-(2-pyridyl)ethyl]-1,3-dihydroisobenzofuran-1-one	0			
AB_0000810	2-phenoxy-2-phenyl-1-ethanol	2	1		0
AB_0000860	1,3-diphenylimidazolidine-2,4-dione	2	2	1	0
AB_0000880	5-bromo-3-phenyl-thiazolidine-2,4-dione	0			
AB_0000900	1-(2-naphthoyl)imidazole	0			
AB_0000910	3-bromo-4-methoxyphenylacetone	2	2	0	0
AB_0000930	3-chloro-1-phenyl-pyrrole-2,5-dione	0			
AB_0000990	3-butyl-2-hydroxy-4h-pyrido[1,2-a]pyrimidin-4-one	0			
AB_0001000	3-(benzylamino)-1,1,1-trifluoro-2-propanol	1	0		
AB_0001010	2-[5-(2-fluorobenzoyl)-2-thienyl]acetonitrile	2	2	2	1
AB_0001020	5-(3,5-difluorobenzyl)-3-(2-thienyl)-1,2,4-oxadiazole	1	0		
AB_0001030	2-chloro-3-(trifluoromethyl)benzaldehyde	1	0		
AB_0001040	2-hydroxy-3-(2-methyl-1-propenyl)-1,4-naphthoquinone	1	1		0
AB_0001060	2-(2-imino-thiazol-3-yl)-1-naphthalen-2-yl-ethanone	2	0		
AB_0001070	imiloxan hydrochloride	1	1		
AB_0001080	2-(benzylthio)-5-methyl-4,5-dihydro-1h-imidazol-3-ium chloride	0			
AB_0001090	n-(3-chlorophenyl)-maleimide	1	0		
AB_0001100	ethyl 4-oxo-1,4-dihydroquinoline-3-carboxylate	0			
AB_0001130	3-amino-n-[2-(methylthio)ethyl]-4-oxo-3,4-dihydroquinazoline-2-carboxamide	0			
AB_0001150	1-[(3,4-dichlorobenzyl)oxy]-1h-imidazole	2	0		
AB_0001170	3-[(2,4-dichlorobenzyl)amino]-1,1,1-trifluoro-2-propanol	0			
AB_0001180	3-oxo-2-phenyl-2,3-dihydro-4-pyridazinecarboxylic acid	0			
AB_0001190	4-[1-(2-phenylethyl)-(1h)-pyrazol-4-yl]pyridine	2	0		
AB_0001200	methyl 1-hydroxy-2-naphthoate	1	0		
AB_0001220	1-(3-trifluoromethyl-phenyl)imidazole	2	0		
AB_0001230	(2-naphthoxy)acetic acid, n-hydroxysuccinimide ester	0			

AB_0001240	methyl 3-(5-chloro-2-methoxyphenyl)-2,3-epoxypropionate	1	1		0
AB_0001250	1-(3,4-dichlorophenyl)-1,3,3-trimethylurea	2	2	1	0
AB_0001260	2-pyridyl 2-(2,3-dihydro-1,4-benzodioxin-2-yl)-1,3-thiazole-4-carbothioate	0			
AB_0001270	1-[(4-chlorobenzyl)amino]-3-(phenylthio)propan-2-ol	1	0		
AB_0001290	3-[(4-chlorophenoxy)methyl]-5-[(2-pyridylthio)methyl]-1,2,4-oxadiazole	2	1		0
AB_0001300	3-(2-thienylcarbonyl)-4h-pyrido[1,2-a]pyrimidin-4-one	1	0		

As regards table 1, one compound (Compound AB_0001010) showed a positive NMR signal even in the absence of target antibody. That compound is likely to be a false positive and was therefore excluded from further analysis. A total of 22 compounds did not show any binding signal in the NMR experiments performed at highest antibody concentration and were thus designated as non-binders. From 23 compounds which showed signal at the highest antibody concentrations a total of 14 did not show any signal at the first dilution of antibody concentration. These compounds were designated as weak binders. Nine compounds showed some kind of signal at the first dilution of antibody concentration and were thus designated as medium to strong binders. Of these, three compounds AB_0000860, AB_0000910 and AB_0001250 showing a clear signal (2 in table 1) were further analysed at a second dilution of antibody concentration (conc 3 in table 1). Whereas compound AB_0000910 did not show any signal at this concentration, both AB_0000860 and AB_0001250 did and were thus confirmed as strong binders.

As regards the structure-activity relationships, the following observations arose from inspection of the structures of the compounds belonging to the three groups of non-binders, weak binders and medium to strong binders. Preferable for binding seems to be the combination of an aromatic part with and aliphatic part with appropriate elements on both parts. Positive for binding for the aro-

matic part is a *meta-* and/or *para-* substituted phenyl ring without heteroatoms in the ring. Especially the presence of nitrogen in the ring seems to influence binding negatively as well as substituents in *ortho* position. Preferable for binding for the aliphatic part are 1) the presence of a tertiary anilinic nitrogen attached to position 1 and 2) (only) one β-keto group attached to position 1, position 1 being the position where the aliphatic part of the ligands is connected to the assumed deepest laying aromatic ring. Preferably a combination of both features like in the N,N-alkylated urea moiety found in the two hits confirmed as strong binders. AB_0000860 posses two aromatic rings differing by their relation to the keto groups of the hydantion ring. This asymmetry gives the molecule a direction, which from the docking analysis agrees with the requirement of only one keto group in a β-position relative to position 1.

The presence of two keto groups in a β-position relative to position 1 disfavours binding. This is in agreement with docking results, where it can be seen that a second keto group would probably be forced into a rather unfavourable hydrophobic environment. Also, larger ring-systems than six member rings (for instance fused rings) seem to have a negative influence for binding. Among the weak binders five compounds were found containing non-substituted phenyl rings and three compounds containing tri-fluoro-methyl groups. It could be speculated that for these compounds the affinity detected may be related to hydrophobic interactions of a rather non-specific type.

Example 2: Directed library

Actions undertaken after the analysis of the NMR screening

From the analysis of the results, two directed libraries centred on the structures of the confirmed strongest binder AB_0001250 were created.

Directed library centred on the structure of AB_0001250

Hit AB_0001250 was one of the hits designated as strong binders. Also, the structure as such offered a potentially attractive synthetic route for varying the substitution pattern of central motif, i.e. the tetra substituted urea, including the introduction of a handle for immobilisation, e.g. to a gel. Therefore, AB_0001250 was chosen as a starting point in the continued development of improvements.

The analysis started with a search for varying the substitution pattern of the aromatic ring. The di-chloro substituted aromatic ring that is present in AB_0001250 does according to the docking fill the available space in an appropriate way in two dimensions but, since that structure is planar, a pocket above the plane of the ring was not filled.

3-chloro-4-methoxyaniline was chosen as the starting point for further synthetic work, since it can be converted into the desired starting material by alkylation of the anilinic nitrogen with methyl iodide.

One option is to have a fluoro-substituent in the *meta* position, in order to favour hydrogen bonding of protein residues. Also for this, a suitable starting material, namely 3-chloro-4-methoxyaniline, is commercially available. The compounds belonging to the designed directed library together with the structure of the original hit AB_0001250 as shown in Figure 3 were subject to docking and NMR screening.

Example 3: Molecular modelling and docking of directed library

The modelling and docking was performed as described above under Materials and Methods. The results from the docking are as follows:

All docked compounds in the directed library with the exception of AB_0001256 resulted in a docked conformation inside the binding pocket, which very closely resembles the position of the docked hit AB_0001250, see Figure 4. In four of the compounds, this is the best-ranked solution. In one of them (AB_0001252), the solution corresponding to the molecule inside the pocket is the second ranked solution. AB_0001256 lacks one of the methyl groups in the tetra substituted urea moiety. Consequently the corresponding amide bond should be more constrained to a planar geometry as compared to the remaining compounds in the library for which such geometry is forbidden because of steric effects between the methyl groups. Apparently, the non-planar geometry is of importance for docking.

The values of the obtained expected energies of binding in kJ/mol are –10, -13, -12, -14 and –14 for AB_000125 (-1) to (-5) respectively. Orthographic plots of the docked hits are shown in Figure 5.

Example 4: NMR screening of directed library

Table 2: Results from NMR screening of the directed library

Concentration code as follows: conc. 1 means 500 and conc 2 100 nM antibody. NMR signal code: 0 no, 1 weak and 2 strong signal, nd means not determined.

ID	Trivial name	Conc 1	Conc 2
AB_0001251	4-(1,3-Dimethyl-3-phenyl-ureido)-butyric acid methyl ester	0	0
AB_0001252	4-[3-(3-Fluoro-4-methoxy -phenyl)-1,3-dimethyl-ureido]-butyric acid methyl ester	1	1
AB_0001253	4-[3-(3-Chloro-4-methoxy -phenyl)-1,3-dimethyl-ureido]-butyric acid methyl ester	2	1
AB_0001255	4-[3-(3,4-Dichloro-phenyl)-1,3-dimethyl-ureido]-butyric acid methyl ester	2	2
AB_0001257	4-[3-(3,4-Dichloro-phenyl)-3-methyl-ureido]-butyric acid methyl ester	2	0
AB_0001258	4-[3-(3,4-Difluoro-phenyl)-1,3-dimethyl-ureido]-butyric acid methyl ester	1	1
AB_0003090	4-[3-(3,4-Dichloro-phenyl)-1,3-dimethyl-ureido]-butyric acid		
AB_0003290	1-[(3,4-Dichloro-phenyl)-methyl-carbamoyl]-pyrrolidine-2-carboxylic acid methyl ester (R-isomer)	2	2

The compounds AB_0001251 through AB_0001259 and AB_0003130 through AB_0003150, shown Table 2, where screened at the two higher antibody concentrations. The result showed that all compounds except AB_0001251 were interacting with the antibody. It was also shown that compound AB_0001255, which is the original compound AB_0001250 with an extension, has the strongest binding of these compounds in the assay. Further, the results also showed that substituents on the aromatic ring are indispensable for binding in

this type of compounds since the only negative result was obtained with the unsubstituted variants AB_0001251 and AB_0003150.

Example 5: General method for attaching ligand to support

Sepharose™ HP (Amersham Biosciences, Uppsala, Sweden) that had previously been derivatised with allyl glycidyl ether was activated with bromine and coupled with hexametylene-diamine according to a standard protocol. The free amine content was determined to 17 µmol/mL gel according to a standard protocol.

2 mL of this gel was transferred to a reaction vessel together with 2 mL of DMF. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.15 mmol) and diisopropyl amine (0.1 mmol) was added and the suspension was put on a shaker at 30 °C. After 5 min. the ligand to be coupled (0.1 mmol) was added and the reaction was allowed to continue for 15 hours.

Thereafter the gel was transferred to a glass filter funnel and washed with a 1:1 mixture of DMF and acetic acid anhydride. The gel was allowed to be in contact with this solution for 30 min. whereafter it was washed with consecutively DMF, water, and 20 % ethanol.

The amount of ligand coupled to the gel was determined with a NMR-method using tri-methoxy benzene as internal reference.

Example 6: Chromatographic characterisation of affinity media containing the ligand AB_0003291 according to the invention

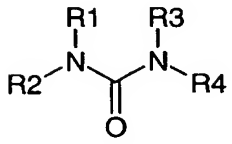
AB_0003291 coupled to Sepharose™ HP (ligand concentration 11 µmol/ml gel as determined by MAS-NMR) was packed in 0.5 ml Tricorn™ 5/20 columns (Amersham Biosciences, Uppsala, Sweden) at a flow rate of 1-2 ml/min. Monoclonals ABFab-K1 (FAB/kappa), ABFab-K2 (Fab'2/kappa) and ABFab-

L2 (Fab/lambda) were tested for binding to AB_0003291-containing gel using protein concentrations of 0.4 or 0.2 mg/ml in PBS, 1 M $(NH_4)_2SO_4$, pH 7. 100 µg of ABFab-K1 and 50 µg of the other two proteins were injected at a flow rate of 0.25 ml/min (contact time 2 min) using an Äkta™ Explorer 10 chromatography system equipped with a UV cell, pH meter, conductivity cell and auto-injector (Amersham Biosciences, Uppsala, Sweden). Protein loading was followed by a wash period of 20 column volumes of loading buffer (PBS, 1 M $(NH_4)_2SO_4$, pH 7) and a second wash step with 0.01 M of NaOH.

Figures 7, 8 and 9 show the separate injections of the monoclonals ABFab-K1, ABFab-K2 and ABFab-L2, respectively, on the AB_0003291-containing media. Evidently, the affinity column containing AB_0003290 coupled to Sepharose™ HP is able to bind the monoclonals ABFab-K1 and ABFab-K2, whereas monoclonal ABFab-L2 directly comes off the affinity column and is found in the flow-through. Elution of monoclonals ABFab-K1 and ABFab-K2 bound to the affinity column in PBS, 1 M $(NH_4)_2SO_4$, pH 7 is possible using different elution conditions such as 50 mM acetate buffer containing 0.14 M NaCl, pH 4 or PBS, pH 7 containing 10% n-propanol (data not shown).

CLAIMS

1. A compound which is represented by formula (I) below



(I)

wherein

R_1 is CH_3 or CH_2CH_3;

R_2 is a *para* and/or *meta* substituted phenyl group;

R_3 is H, CH_3 or CH_2CH_3; and

R_4 is a linear or cyclic aliphatic group, which is optionally substituted,

or, wherein

R_1 and R_2 are as stated above while R_3 and R_4 are parts of a 4- to 6-membered cyclic entity, which is optionally substituted,

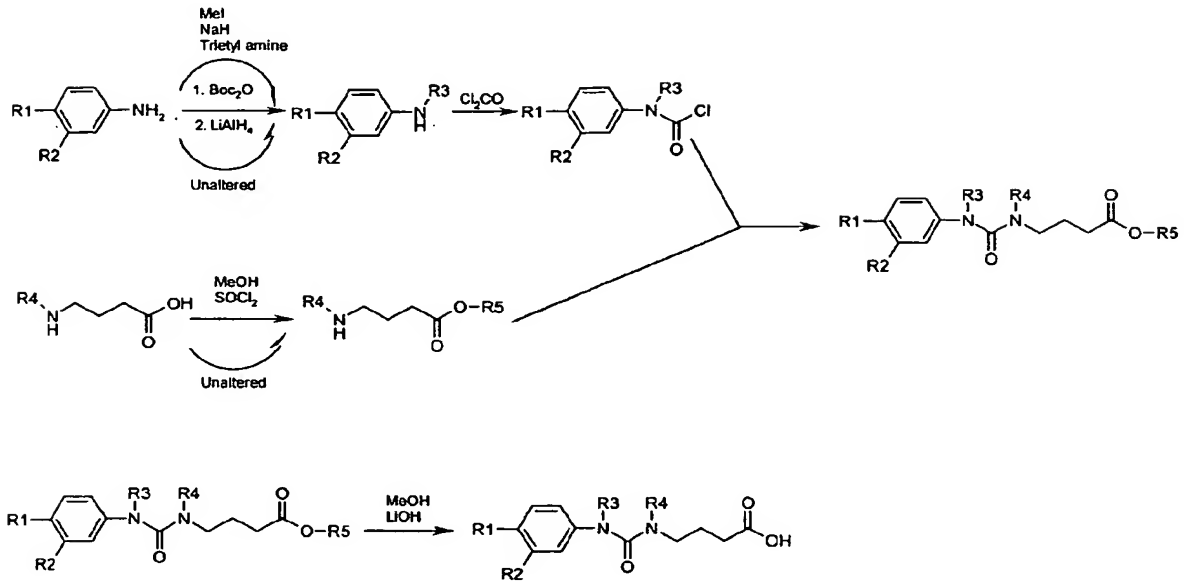
and which compound has affinity for human IgG of κ-type.

2. A compound according to claim 1, which is an affinity ligand with affinity for the constant region of a Fab fragment of human IgG of κ-type.
3. A compound according to claim 1 or 2, wherein R_1 is CH_3.
4. A compound according to any one of the preceding claims, wherein R_2 comprises a substituted phenyl group and the substituents are selected from the group that consists of F, Cl, Br, I and O.
5. A compound according to any one of the preceding claims, wherein the phenyl group of R_2 is substituted in the *para* position with a group defined as $-O-R_5$, wherein R_5 is either CH_3 or CH_2CH_3.
6. A compound according to any one of the preceding claims, wherein the phenyl group of R_2 is substituted with Cl or F in the *meta* position.

7. A compound according to any one of claims 1-4, wherein the phenyl group of R_2 is substituted with Cl in *meta* and *para* position.
8. A compound according to any one of the preceding claims, wherein R_4 is an aliphatic group, which is interrupted in one or more positions by oxygen atoms.
9. A compound according to any one of the preceding claims, wherein R_4 is an aliphatic group, which comprises one or more carbonyl group.
10. A compound according to any one of the preceding claims, wherein R_4 is an aliphatic group that comprises a terminating functionality selected from the group that consists of a carboxylic acid, nitrogen, oxygen, sulphur or any derivative thereof.
11. A compound according to any one of the preceding claims, wherein R_1 is CH_3; R_2 is a phenyl group that has been substituted with Cl in *meta* and *para* position; and R_3 and R_4 are parts of a cyclic 5-membered group, which is optionally substituted.
12. A compound according to claim 11, wherein the cyclic 5-membered entity is substituted in a position directly adjacent to N with a C(O)-O-CH3 group.
13. A compound according to any one of the preceding claims, which is capable of binding human to the constant region of IgG of κ-type, or a functional derivative thereof, with a binding constant of at least 10^{-3} M.
14. A compound according to any one of the preceding claims, which is capable of binding to the constant region of a human IgG of κ-type, or a functional derivative thereof, via a binding pocket defined by the structure coordinates of the amino acids as shown in Fig 6.
15. Use of a compound according to any one of claims 1-14 for selective binding to the constant region of human IgG of κ-type, or a functional derivative thereof.
16. A sorption complex comprised of a compound according to any one of claims 1-14 directly linked to the constant region of a Fab fragment of a human IgG of κ-type, or a functional derivative thereof.

17. A separation matrix for affinity chromatography, which matrix comprises ligands coupled to a support, wherein the majority of the ligands are compounds as defined in any one of claims 1-14.
18. A separation matrix according to claim 17, wherein the ligands have been coupled to the support via linkers.
19. A separation matrix according to claim 17 or 18, wherein the support is a porous polymeric particle.
20. A generic method of isolating human IgG of κ-type from other components in a liquid, wherein a compound as defined in any one of claims 1-14 or a separation matrix according to any one of claims 17-19 is used.
21. A system suitable for affinity chromatography, which is comprised of a separation matrix as defined in any one of claims 17-19 packed in a column.

Figure 1: Synthetic route to variations



R1 = Cl, F, OMe
R2 = Cl, F
R3 = H, Me
R4 = H, Me
R5 = H, Me

Figure 2

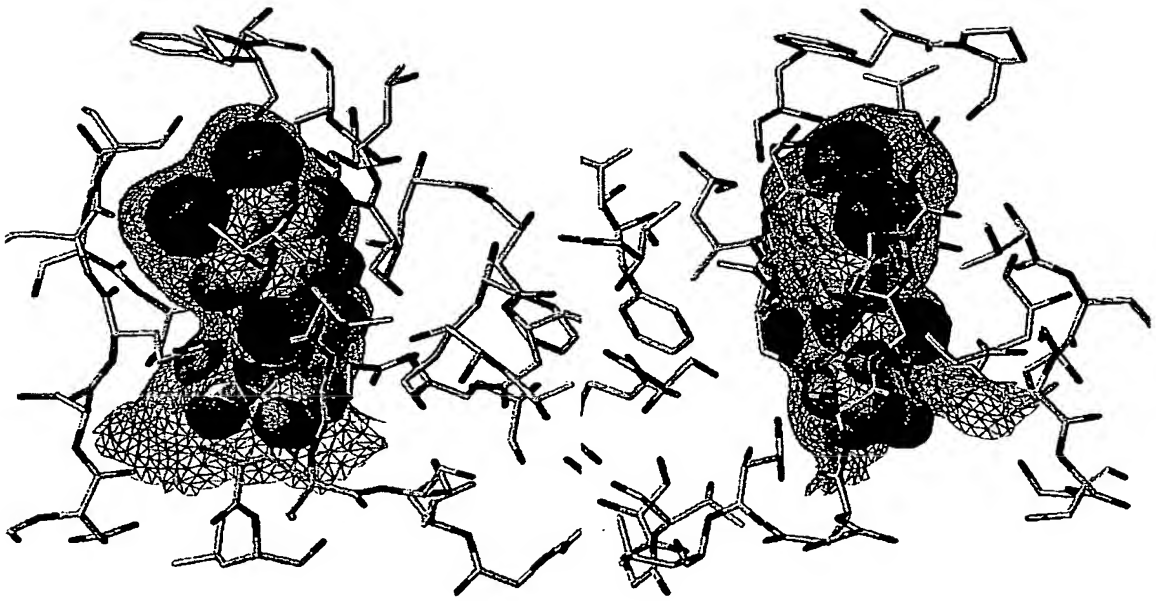


Figure 3: Illustrative compounds

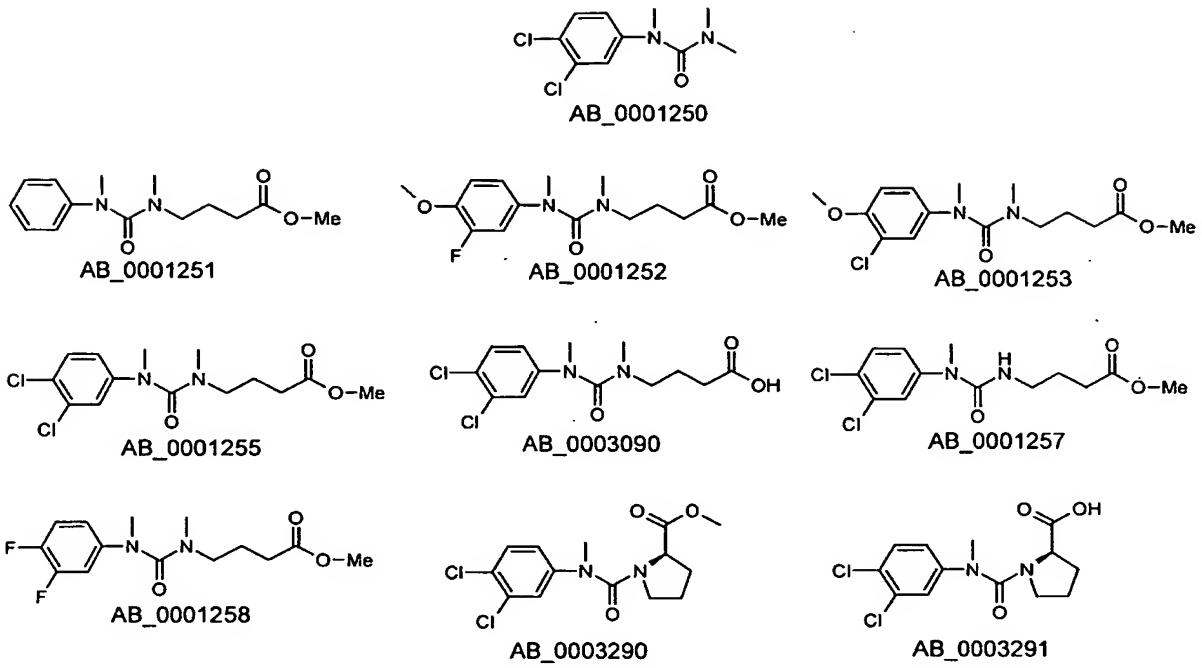


Figure 4: Orthographic views

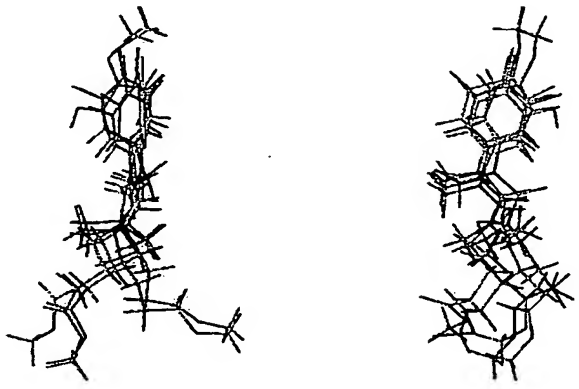


Figure 5A-E: Orthographic views of the docked compounds AB_000125[1-5].

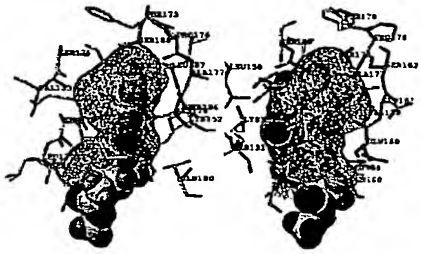


Fig 5A: AB_0001251

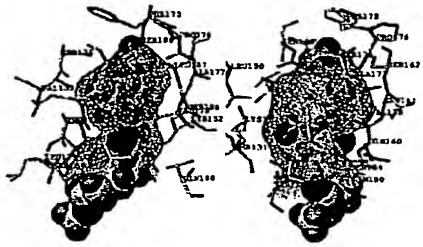


Fig 5B: AB_0001252

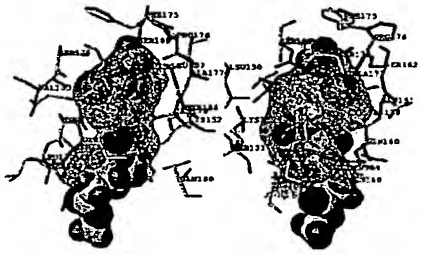


Fig 5C: AB_0001253

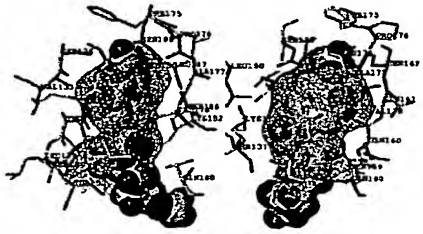


Fig 5D: AB_0001254

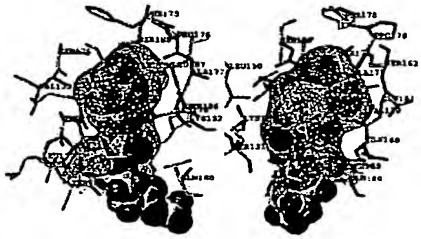


Fig 5E: AB_0001255

Fig 6a kappa light chain

ATOM	928	N	GLN	L	124	-44.718	27.024	79.393	1.00	37.64	N
ATOM	929	CA	GLN	L	124	-43.847	25.897	79.535	1.00	38.32	C
ATOM	930	C	GLN	L	124	-44.309	25.088	80.734	1.00	39.17	C
ATOM	931	O	GLN	L	124	-44.458	23.876	80.578	1.00	40.06	O
ATOM	932	CB	GLN	L	124	-42.414	26.311	79.745	1.00	37.76	C
ATOM	933	CG	GLN	L	124	-41.615	25.026	79.581	1.00	34.56	C
ATOM	934	CD	GLN	L	124	-40.133	25.152	79.698	1.00	34.95	C
ATOM	935	OE1	GLN	L	124	-39.440	24.138	79.682	1.00	34.80	O
ATOM	936	NE2	GLN	L	124	-39.569	26.344	79.820	1.00	39.75	N
ATOM	954	N	SER	L	127	-46.898	22.499	80.067	1.00	50.58	N
ATOM	955	CA	SER	L	127	-46.559	21.169	79.588	1.00	49.80	C
ATOM	956	C	SER	L	127	-45.890	20.274	80.637	1.00	49.81	C
ATOM	957	O	SER	L	127	-45.283	19.248	80.318	1.00	50.44	O
ATOM	958	CB	SER	L	127	-45.674	21.333	78.368	1.00	50.26	C
ATOM	959	OG	SER	L	127	-44.618	22.263	78.551	1.00	51.43	O
ATOM	960	N	GLY	L	128	-45.954	20.623	81.919	1.00	48.65	N
ATOM	961	CA	GLY	L	128	-45.371	19.786	82.925	1.00	47.11	C
ATOM	962	C	GLY	L	128	-43.851	19.873	82.985	1.00	46.88	C
ATOM	963	O	GLY	L	128	-43.322	19.013	83.700	1.00	46.88	O
ATOM	964	N	THR	L	129	-43.091	20.805	82.358	1.00	46.66	N
ATOM	965	CA	THR	L	129	-41.625	20.919	82.516	1.00	43.85	C
ATOM	966	C	THR	L	129	-41.246	22.341	82.832	1.00	37.58	C
ATOM	967	O	THR	L	129	-42.031	23.269	82.637	1.00	35.77	O
ATOM	968	CB	THR	L	129	-40.785	20.528	81.250	1.00	48.03	C
ATOM	969	OG1	THR	L	129	-41.566	20.726	80.058	1.00	54.58	O
ATOM	970	CG2	THR	L	129	-40.269	19.111	81.408	1.00	49.23	C
ATOM	976	N	SER	L	131	-37.741	24.856	82.399	1.00	29.71	N
ATOM	977	CA	SER	L	131	-36.337	25.100	82.108	1.00	27.40	C
ATOM	978	C	SER	L	131	-35.958	26.455	82.672	1.00	24.38	C
ATOM	979	O	SER	L	131	-36.663	27.454	82.446	1.00	23.59	O
ATOM	980	CB	SER	L	131	-36.097	25.078	80.593	1.00	29.26	C
ATOM	981	OG	SER	L	131	-36.672	23.985	79.880	1.00	28.43	O
ATOM	989	N	VAL	L	133	-32.859	29.248	82.770	1.00	23.53	N
ATOM	990	CA	VAL	L	133	-31.671	29.552	81.985	1.00	21.58	C
ATOM	991	C	VAL	L	133	-30.829	30.592	82.700	1.00	21.93	C
ATOM	992	O	VAL	L	133	-31.363	31.514	83.297	1.00	22.42	O
ATOM	993	CB	VAL	L	133	-32.042	30.112	80.607	1.00	21.06	C
ATOM	994	CG1	VAL	L	133	-30.831	30.026	79.693	1.00	25.56	C
ATOM	995	CG2	VAL	L	133	-33.149	29.296	79.958	1.00	24.10	C
ATOM	1188	N	GLY	L	157	-26.853	18.788	90.054	1.00	53.00	N
ATOM	1189	CA	GLY	L	157	-26.116	18.154	88.943	1.00	52.14	C
ATOM	1190	C	GLY	L	157	-27.023	17.720	87.749	1.00	51.94	C
ATOM	1191	O	GLY	L	157	-26.809	16.631	87.208	1.00	52.06	O
ATOM	1192	N	ASN	L	158	-28.025	18.503	87.273	1.00	50.46	N
ATOM	1193	CA	ASN	L	158	-28.946	18.183	86.142	1.00	46.46	C
ATOM	1194	C	ASN	L	158	-29.116	19.347	85.106	1.00	44.90	C
ATOM	1195	O	ASN	L	158	-30.222	19.704	84.625	1.00	40.45	O
ATOM	1196	CB	ASN	L	158	-30.312	17.839	86.692	1.00	47.64	C
ATOM	1197	CG	ASN	L	158	-30.916	19.055	87.386	1.00	52.21	C
ATOM	1198	OD1	ASN	L	158	-30.438	19.529	88.430	1.00	47.79	O
ATOM	1199	ND2	ASN	L	158	-31.930	19.646	86.768	1.00	55.19	N
ATOM	1200	N	SER	L	159	-27.980	19.972	84.739	1.00	41.19	N
ATOM	1201	CA	SER	L	159	-27.943	21.083	83.811	1.00	38.82	C
ATOM	1202	C	SER	L	159	-27.136	20.769	82.554	1.00	38.54	C
ATOM	1203	O	SER	L	159	-26.262	19.891	82.610	1.00	39.32	O
ATOM	1204	CB	SER	L	159	-27.323	22.257	84.505	1.00	32.87	C
ATOM	1205	OG	SER	L	159	-26.007	21.915	84.912	1.00	34.12	O
ATOM	1206	N	GLN	L	160	-27.397	21.485	81.451	1.00	37.38	N
ATOM	1207	CA	GLN	L	160	-26.575	21.389	80.266	1.00	35.88	C
ATOM	1208	C	GLN	L	160	-26.118	22.789	79.886	1.00	32.74	C
ATOM	1209	O	GLN	L	160	-26.831	23.765	80.112	1.00	28.45	O
ATOM	1210	CB	GLN	L	160	-27.325	20.798	79.077	1.00	40.64	C
ATOM	1211	CG	GLN	L	160	-27.352	19.273	79.129	1.00	47.64	C
ATOM	1212	CD	GLN	L	160	-27.353	18.619	77.751	1.00	51.42	C
ATOM	1213	OE1	GLN	L	160	-26.474	17.841	77.354	1.00	54.61	O

Fig 6a kappa light chain

ATOM	1214	NE2	GLN	L	160	-28.351	18.941	76.956	1.00	51.87	N
ATOM	1215	N	GLU	L	161	-24.947	22.884	79.252	1.00	32.26	N
ATOM	1216	CA	GLU	L	161	-24.315	24.116	78.812	1.00	30.57	C
ATOM	1217	C	GLU	L	161	-24.096	24.228	77.315	1.00	29.51	C
ATOM	1218	O	GLU	L	161	-24.030	23.218	76.609	1.00	31.47	O
ATOM	1219	CB	GLU	L	161	-22.989	24.254	79.465	1.00	31.63	C
ATOM	1220	CG	GLU	L	161	-23.068	25.232	80.584	1.00	39.52	C
ATOM	1221	CD	GLU	L	161	-22.438	24.715	81.857	1.00	45.11	C
ATOM	1222	OE1	GLU	L	161	-21.196	24.764	81.949	1.00	43.57	O
ATOM	1223	OE2	GLU	L	161	-23.211	24.287	82.736	1.00	48.88	O
ATOM	1224	N	SER	L	162	-23.964	25.449	76.818	1.00	27.95	N
ATOM	1225	CA	SER	L	162	-23.733	25.712	75.415	1.00	24.52	C
ATOM	1226	C	SER	L	162	-22.917	27.003	75.355	1.00	23.12	C
ATOM	1227	O	SER	L	162	-23.213	27.968	76.057	1.00	21.32	O
ATOM	1228	CB	SER	L	162	-25.089	25.831	74.776	1.00	24.91	C
ATOM	1229	OG	SER	L	162	-24.944	26.008	73.380	1.00	28.23	O
ATOM	1332	N	SER	L	176	-24.700	29.533	78.016	1.00	20.73	N
ATOM	1333	CA	SER	L	176	-25.984	29.359	78.650	1.00	20.18	C
ATOM	1334	C	SER	L	176	-25.967	28.050	79.391	1.00	19.90	C
ATOM	1335	O	SER	L	176	-25.400	27.058	78.938	1.00	18.83	O
ATOM	1336	CB	SER	L	176	-27.081	29.343	77.602	1.00	22.81	C
ATOM	1337	OG	SER	L	176	-26.755	28.427	76.557	1.00	27.50	O
ATOM	1338	N	SER	L	177	-26.543	28.045	80.570	1.00	21.10	N
ATOM	1339	CA	SER	L	177	-26.716	26.843	81.325	1.00	22.83	C
ATOM	1340	C	SER	L	177	-28.233	26.701	81.427	1.00	24.50	C
ATOM	1341	O	SER	L	177	-28.927	27.679	81.752	1.00	26.47	O
ATOM	1342	CB	SER	L	177	-26.100	27.030	82.675	1.00	20.36	C
ATOM	1343	OG	SER	L	177	-25.923	25.738	83.209	1.00	25.00	O
ATOM	1344	N	THR	L	178	-28.783	25.535	81.113	1.00	26.21	N
ATOM	1345	CA	THR	L	178	-30.193	25.289	81.284	1.00	25.67	C
ATOM	1346	C	THR	L	178	-30.333	24.182	82.316	1.00	26.52	C
ATOM	1347	O	THR	L	178	-29.692	23.127	82.251	1.00	25.41	O
ATOM	1348	CB	THR	L	178	-30.797	24.854	79.993	1.00	24.43	C
ATOM	1349	OG1	THR	L	178	-30.504	25.890	79.065	1.00	27.73	O
ATOM	1350	CG2	THR	L	178	-32.288	24.606	80.101	1.00	23.92	C
ATOM	1359	N	THR	L	180	-33.064	21.776	83.928	1.00	33.72	N
ATOM	1360	CA	THR	L	180	-34.412	21.334	83.617	1.00	36.96	C
ATOM	1361	C	THR	L	180	-34.895	20.441	84.742	1.00	39.75	C
ATOM	1362	O	THR	L	180	-34.162	19.554	85.220	1.00	40.12	O
ATOM	1363	CB	THR	L	180	-34.439	20.578	82.248	1.00	37.34	C
ATOM	1364	OG1	THR	L	180	-34.262	21.580	81.236	1.00	38.56	O
ATOM	1365	CG2	THR	L	180	-35.746	19.829	81.975	1.00	36.31	C
ATOM	1366	N	LEU	L	181	-36.102	20.772	85.213	1.00	41.45	N
ATOM	1367	CA	LEU	L	181	-36.790	19.955	86.189	1.00	41.68	C
ATOM	1368	C	LEU	L	181	-38.283	19.907	85.844	1.00	41.64	C
ATOM	1369	O	LEU	L	181	-38.823	20.667	85.022	1.00	39.32	O
ATOM	1370	CB	LEU	L	181	-36.472	20.527	87.616	1.00	41.26	C
ATOM	1371	CG	LEU	L	181	-36.887	21.835	88.321	1.00	44.99	C
ATOM	1372	CD1	LEU	L	181	-35.940	21.997	89.487	1.00	42.76	C
ATOM	1373	CD2	LEU	L	181	-36.694	23.093	87.505	1.00	45.40	C

Fig 6b kappa heavy chain

ATOM	2595	N	LYS	H	126	-39.678	16.046	64.413	1.00	20.92	N
ATOM	2596	CA	LYS	H	126	-40.480	16.635	65.460	1.00	19.79	C
ATOM	2597	C	LYS	H	126	-40.194	18.131	65.371	1.00	21.17	C
ATOM	2598	O	LYS	H	126	-39.025	18.554	65.306	1.00	23.97	O
ATOM	2599	CB	LYS	H	126	-40.054	16.081	66.825	1.00	18.88	C
ATOM	2600	CG	LYS	H	126	-41.090	16.590	67.794	1.00	26.44	C
ATOM	2601	CD	LYS	H	126	-40.944	16.341	69.291	1.00	32.32	C
ATOM	2602	CE	LYS	H	126	-41.916	17.243	70.134	1.00	37.31	C
ATOM	2603	NZ	LYS	H	126	-41.584	18.677	70.172	1.00	35.44	N
ATOM	2608	N	PRO	H	128	-40.310	22.204	66.796	1.00	18.58	N
ATOM	2609	CA	PRO	H	128	-39.950	22.699	68.117	1.00	19.70	C
ATOM	2610	C	PRO	H	128	-41.041	23.367	68.948	1.00	22.10	C
ATOM	2611	O	PRO	H	128	-42.127	23.691	68.475	1.00	25.30	O
ATOM	2612	CB	PRO	H	128	-38.769	23.602	67.812	1.00	18.78	C
ATOM	2613	CG	PRO	H	128	-39.053	24.200	66.457	1.00	17.91	C
ATOM	2614	CD	PRO	H	128	-39.898	23.122	65.749	1.00	20.81	C
ATOM	2615	N	SER	H	129	-40.828	23.620	70.221	1.00	24.82	N
ATOM	2616	CA	SER	H	129	-41.770	24.395	70.995	1.00	23.50	C
ATOM	2617	C	SER	H	129	-40.946	25.623	71.266	1.00	24.48	C
ATOM	2618	O	SER	H	129	-39.763	25.502	71.565	1.00	22.58	O
ATOM	2619	CB	SER	H	129	-42.105	23.686	72.286	1.00	28.31	C
ATOM	2620	OG	SER	H	129	-42.934	22.546	72.073	1.00	36.78	O
ATOM	2628	N	PHE	H	131	-40.521	28.925	73.626	1.00	30.04	N
ATOM	2629	CA	PHE	H	131	-41.040	29.482	74.848	1.00	27.87	C
ATOM	2630	C	PHE	H	131	-40.215	30.723	75.051	1.00	30.92	C
ATOM	2631	O	PHE	H	131	-39.007	30.683	74.789	1.00	26.23	O
ATOM	2632	CB	PHE	H	131	-40.810	28.570	76.022	1.00	30.11	C
ATOM	2633	CG	PHE	H	131	-41.537	27.235	75.970	1.00	30.98	C
ATOM	2634	CD1	PHE	H	131	-42.931	27.183	75.945	1.00	30.57	C
ATOM	2635	CD2	PHE	H	131	-40.808	26.050	75.966	1.00	33.12	C
ATOM	2636	CE1	PHE	H	131	-43.590	25.948	75.915	1.00	31.13	C
ATOM	2637	CE2	PHE	H	131	-41.479	24.815	75.932	1.00	33.57	C
ATOM	2638	CZ	PHE	H	131	-42.863	24.765	75.907	1.00	31.34	C
ATOM	2646	N	LEU	H	133	-38.146	33.716	77.032	1.00	38.18	N
ATOM	2647	CA	LEU	H	133	-37.285	33.930	78.190	1.00	34.16	C
ATOM	2648	C	LEU	H	133	-37.523	35.428	78.330	1.00	35.44	C
ATOM	2649	O	LEU	H	133	-37.005	36.294	77.609	1.00	32.35	O
ATOM	2650	CB	LEU	H	133	-35.823	33.622	77.863	1.00	29.24	C
ATOM	2651	CG	LEU	H	133	-35.533	32.258	77.309	1.00	22.14	C
ATOM	2652	CD1	LEU	H	133	-34.066	32.136	77.012	1.00	23.67	C
ATOM	2653	CD2	LEU	H	133	-35.970	31.213	78.300	1.00	27.77	C
ATOM	2749	N	LEU	H	150	-36.371	30.246	73.846	1.00	22.90	N
ATOM	2750	CA	LEU	H	150	-35.971	28.876	74.075	1.00	23.38	C
ATOM	2751	C	LEU	H	150	-36.705	28.058	73.003	1.00	25.45	C
ATOM	2752	O	LEU	H	150	-37.917	28.204	72.817	1.00	24.96	O
ATOM	2753	CB	LEU	H	150	-36.391	28.505	75.477	1.00	18.99	C
ATOM	2754	CG	LEU	H	150	-36.325	27.052	75.868	1.00	19.75	C
ATOM	2755	CD1	LEU	H	150	-34.917	26.528	75.789	1.00	22.45	C
ATOM	2756	CD2	LEU	H	150	-36.781	26.912	77.286	1.00	19.55	C
ATOM	2764	N	LYS	H	152	-37.287	24.376	72.183	1.00	25.67	N
ATOM	2765	CA	LYS	H	152	-37.209	23.103	72.858	1.00	23.11	C
ATOM	2766	C	LYS	H	152	-37.793	21.909	72.110	1.00	23.19	C
ATOM	2767	O	LYS	H	152	-38.886	21.985	71.563	1.00	22.11	O
ATOM	2768	CB	LYS	H	152	-37.905	23.319	74.200	1.00	25.76	C
ATOM	2769	CG	LYS	H	152	-37.302	22.378	75.195	1.00	29.14	C
ATOM	2770	CD	LYS	H	152	-37.759	22.579	76.622	1.00	30.74	C
ATOM	2771	CE	LYS	H	152	-36.922	21.597	77.460	1.00	28.69	C
ATOM	2772	NZ	LYS	H	152	-37.314	20.228	77.199	1.00	25.73	N
ATOM	2773	N	ASP	H	153	-37.045	20.807	72.047	1.00	25.81	N
ATOM	2774	CA	ASP	H	153	-37.461	19.487	71.575	1.00	22.60	C
ATOM	2775	C	ASP	H	153	-37.870	19.231	70.146	1.00	20.15	C
ATOM	2776	O	ASP	H	153	-38.939	18.761	69.803	1.00	18.56	O
ATOM	2777	CB	ASP	H	153	-38.561	19.010	72.523	1.00	26.65	C
ATOM	2778	CG	ASP	H	153	-38.083	18.807	73.962	1.00	26.68	C
ATOM	2779	OD1	ASP	H	153	-36.935	18.446	74.194	1.00	28.52	O
ATOM	2780	OD2	ASP	H	153	-38.866	19.018	74.873	1.00	26.88	O

Fig 6b kappa heavy chain

ATOM	2940	N	PHE	H	175	-27.214	30.210	70.335	1.00	23.94	N
ATOM	2941	CA	PHE	H	175	-26.383	29.122	70.813	1.00	23.42	C
ATOM	2942	C	PHE	H	175	-26.478	27.831	69.986	1.00	23.74	C
ATOM	2943	O	PHE	H	175	-27.538	27.522	69.409	1.00	23.81	O
ATOM	2944	CB	PHE	H	175	-26.758	28.815	72.248	1.00	22.94	C
ATOM	2945	CG	PHE	H	175	-26.259	29.899	73.148	1.00	20.21	C
ATOM	2946	CD1	PHE	H	175	-24.971	29.801	73.645	1.00	19.49	C
ATOM	2947	CD2	PHE	H	175	-27.079	30.977	73.458	1.00	20.84	C
ATOM	2948	CE1	PHE	H	175	-24.497	30.807	74.468	1.00	20.36	C
ATOM	2949	CE2	PHE	H	175	-26.595	31.980	74.294	1.00	22.58	C
ATOM	2950	CZ	PHE	H	175	-25.300	31.901	74.800	1.00	21.02	C
ATOM	2951	N	PRO	H	176	-25.360	27.078	69.878	1.00	22.56	N
ATOM	2952	CA	PRO	H	176	-25.321	25.723	69.318	1.00	19.83	C
ATOM	2953	C	PRO	H	176	-26.377	24.835	69.977	1.00	21.20	C
ATOM	2954	O	PRO	H	176	-26.508	24.942	71.200	1.00	22.55	O
ATOM	2955	CB	PRO	H	176	-23.910	25.305	69.595	1.00	16.67	C
ATOM	2956	CG	PRO	H	176	-23.083	26.559	69.637	1.00	15.02	C
ATOM	2957	CD	PRO	H	176	-24.018	27.503	70.334	1.00	17.20	C
ATOM	2963	N	VAL	H	178	-28.150	21.582	71.822	1.00	22.66	N
ATOM	2964	CA	VAL	H	178	-27.623	20.460	72.565	1.00	21.24	C
ATOM	2965	C	VAL	H	178	-28.654	19.371	72.365	1.00	20.88	C
ATOM	2966	O	VAL	H	178	-29.868	19.553	72.269	1.00	22.54	O
ATOM	2967	CB	VAL	H	178	-27.441	20.749	74.109	1.00	23.34	C
ATOM	2968	CG1	VAL	H	178	-26.426	21.863	74.326	1.00	21.50	C
ATOM	2969	CG2	VAL	H	178	-28.744	21.171	74.737	1.00	25.02	C
ATOM	2970	N	LEU	H	179	-28.110	18.208	72.193	1.00	22.43	N
ATOM	2971	CA	LEU	H	179	-28.876	17.011	72.085	1.00	25.70	C
ATOM	2972	C	LEU	H	179	-29.097	16.527	73.522	1.00	25.97	C
ATOM	2973	O	LEU	H	179	-28.187	16.399	74.348	1.00	25.39	O
ATOM	2974	CB	LEU	H	179	-28.076	16.026	71.278	1.00	25.57	C
ATOM	2975	CG	LEU	H	179	-28.702	14.674	71.023	1.00	27.43	C
ATOM	2976	CD1	LEU	H	179	-29.897	14.757	70.074	1.00	19.28	C
ATOM	2977	CD2	LEU	H	179	-27.587	13.805	70.469	1.00	30.31	C
ATOM	2978	N	GLN	H	180	-30.365	16.320	73.815	1.00	27.28	N
ATOM	2979	CA	GLN	H	180	-30.821	15.886	75.111	1.00	25.86	C
ATOM	2980	C	GLN	H	180	-30.787	14.360	75.199	1.00	26.76	C
ATOM	2981	O	GLN	H	180	-30.630	13.675	74.180	1.00	27.19	O
ATOM	2982	CB	GLN	H	180	-32.233	16.463	75.292	1.00	28.23	C
ATOM	2983	CG	GLN	H	180	-32.316	17.984	75.105	1.00	28.44	C
ATOM	2984	CD	GLN	H	180	-33.725	18.562	75.115	1.00	31.65	C
ATOM	2985	OE1	GLN	H	180	-34.406	18.608	74.093	1.00	30.70	O
ATOM	2986	NE2	GLN	H	180	-34.230	19.012	76.261	1.00	30.98	N
ATOM	2987	N	SER	H	181	-30.940	13.753	76.391	1.00	28.39	N
ATOM	2988	CA	SER	H	181	-30.945	12.305	76.549	1.00	28.80	C
ATOM	2989	C	SER	H	181	-32.113	11.663	75.787	1.00	25.40	C
ATOM	2990	O	SER	H	181	-31.965	10.542	75.300	1.00	28.76	O
ATOM	2991	CB	SER	H	181	-30.979	12.001	78.067	1.00	31.94	C
ATOM	2992	OG	SER	H	181	-31.812	12.915	78.815	1.00	40.94	O
ATOM	2993	N	SER	H	182	-33.258	12.324	75.579	1.00	21.90	N
ATOM	2994	CA	SER	H	182	-34.325	11.787	74.720	1.00	24.38	C
ATOM	2995	C	SER	H	182	-33.959	11.687	73.227	1.00	25.28	C
ATOM	2996	O	SER	H	182	-34.562	10.902	72.497	1.00	29.85	O
ATOM	2997	CB	SER	H	182	-35.556	12.654	74.850	1.00	17.40	C
ATOM	2998	OG	SER	H	182	-35.104	13.995	74.772	1.00	19.22	O
ATOM	3003	N	LEU	H	184	-33.775	14.556	71.267	1.00	20.48	N
ATOM	3004	CA	LEU	H	184	-34.278	15.749	70.637	1.00	17.63	C
ATOM	3005	C	LEU	H	184	-33.314	16.869	71.000	1.00	18.68	C
ATOM	3006	O	LEU	H	184	-32.549	16.765	71.956	1.00	16.48	O
ATOM	3007	CB	LEU	H	184	-35.675	15.980	71.168	1.00	18.23	C
ATOM	3008	CG	LEU	H	184	-36.724	14.864	71.080	1.00	12.53	C
ATOM	3009	CD1	LEU	H	184	-37.909	15.249	71.922	1.00	10.58	C
ATOM	3010	CD2	LEU	H	184	-37.141	14.621	69.658	1.00	13.49	C
ATOM	3023	N	SER	H	186	-32.310	21.176	71.626	1.00	19.45	N
ATOM	3024	CA	SER	H	186	-32.755	22.411	72.223	1.00	20.48	C
ATOM	3025	C	SER	H	186	-31.701	23.450	71.937	1.00	23.05	C
ATOM	3026	O	SER	H	186	-30.521	23.102	71.874	1.00	25.18	O
ATOM	3027	CB	SER	H	186	-32.916	22.306	73.718	1.00	21.58	C
ATOM	3028	OG	SER	H	186	-34.253	21.920	74.021	1.00	32.73	O

Fig 6b kappa heavy chain

ATOM	3029	N	LEU	H	187	-32.104	24.707	71.768	1.00 21.56	N
ATOM	3030	CA	LEU	H	187	-31.233	25.811	71.415	1.00 21.58	C
ATOM	3031	C	LEU	H	187	-31.765	27.082	72.120	1.00 23.47	C
ATOM	3032	O	LEU	H	187	-32.948	27.118	72.496	1.00 24.42	O
ATOM	3033	CB	LEU	H	187	-31.309	25.838	69.897	1.00 19.86	C
ATOM	3034	CG	LEU	H	187	-30.875	26.971	69.054	1.00 21.75	C
ATOM	3035	CD1	LEU	H	187	-30.413	26.485	67.691	1.00 19.38	C
ATOM	3036	CD2	LEU	H	187	-32.048	27.868	68.864	1.00 23.32	C
ATOM	3037	N	SER	H	188	-31.014	28.142	72.424	1.00 22.73	N
ATOM	3038	CA	SER	H	188	-31.587	29.401	72.873	1.00 21.20	C
ATOM	3039	C	SER	H	188	-31.069	30.509	71.988	1.00 20.80	C
ATOM	3040	O	SER	H	188	-29.961	30.400	71.441	1.00 21.00	O
ATOM	3041	CB	SER	H	188	-31.179	29.775	74.274	1.00 25.10	C
ATOM	3042	OG	SER	H	188	-31.586	28.721	75.127	1.00 31.30	O

Figure 7

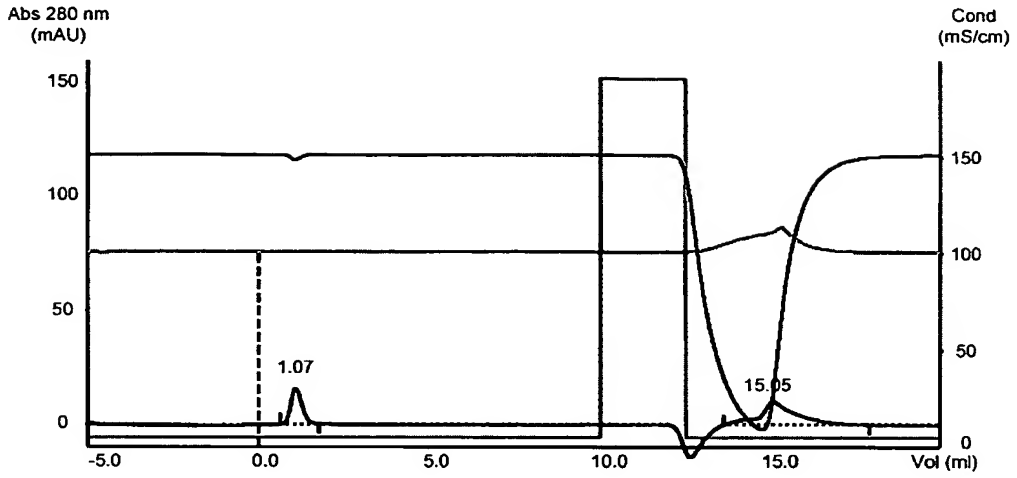


Figure 8

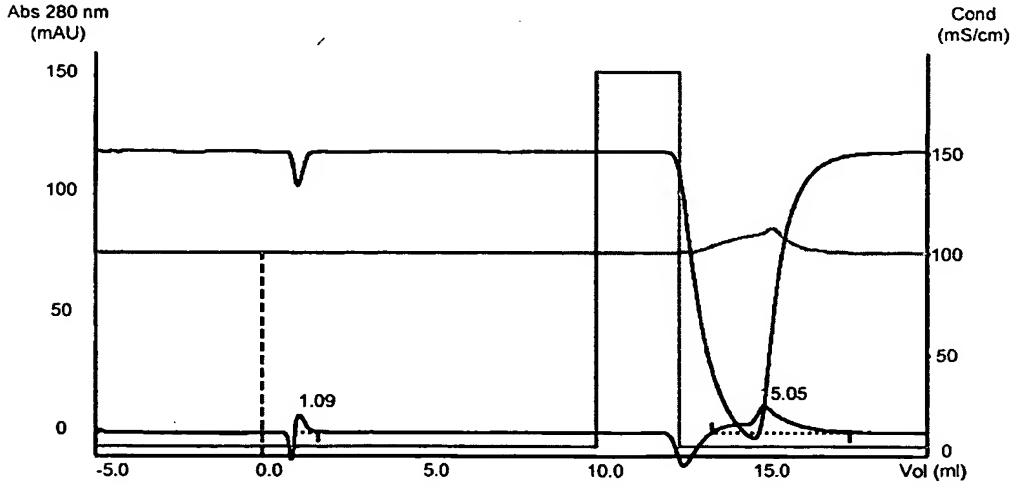
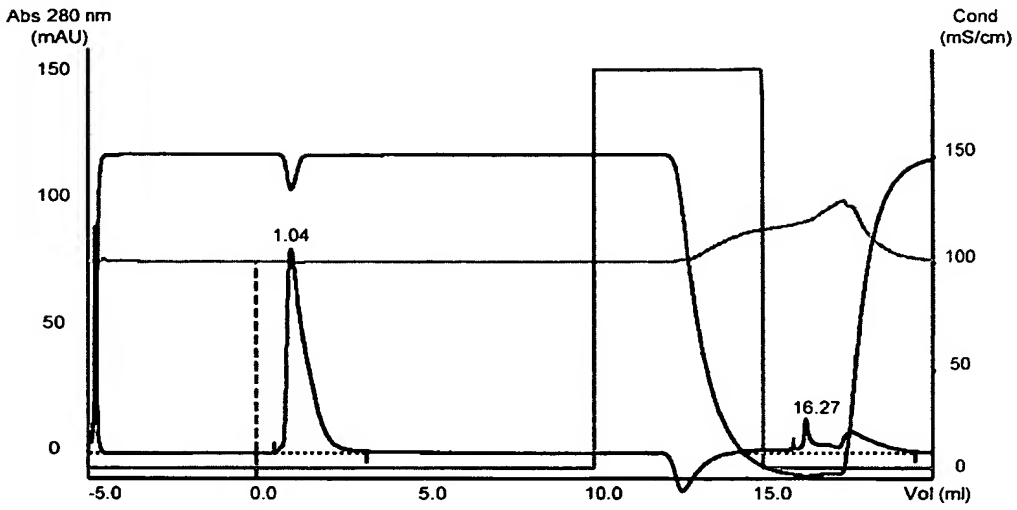


Figure 9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Cond
(mS/cm)
150
100
50
0
1.04
16.27
-5.0
0.0
5.0
10.0
15.0
Vol (ml)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 03/01434

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07C 275/00, C07D 213/81, A61P 37/00, C07D 233/34, A61K 31/17, C12Q 1/58, G01N 33/62 // C08G 71/02, A01N 47/28, C07K 16/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07C, C07D, A61K, C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, STN, CAPLUS, EMBASE, PAJ, EPODOC, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	J. Med. Chem., Volume 46, 2003, Nathan R. McElroy et al: 2QSAR and Classification of Murine and Human Soluble Epoxide Hydrolase Inhibition by Urea-Like Compounds", page 1066 - page 1080, table I, II --	1-14
X	J. Microcolumn Separations, Volume 12, no. 5, 2000, Eric Schoenzetter et al: "Rapid Sample Handling in Microcolumn-Liquid Chromatography Using Selective On-Line Immunoaffinity Extraction", page 316 - page 322, figure 1, table 1 --	1-14,16-19, 21

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 December 2003	15 -12- 2003
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Fernando Farieta/EÖ Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 03/01434

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Chromatography A, Volume 732, 1996, James F. Lawrence et al: "Use of immunoaffinity chromatography as a simplified cleanup technique for the liquid chromatographic determination of phenylurea herbicides in plant material", page 277 - page 281, figure 1	1-14,16-19, 21
X	J. Agric. Food Chem., Volume 42, 1994, Peter Schneider et al: "A Highly Sensitive and Rapid ELISA for the Arylurea Herbicides Diuron, Monuron, and Linuron", page 413 - page 422, figure 1, compounds 6-10	1-14
A		15-19,21
X	WO 9727752 A1 (MERCK & CO., INC.), 7 August 1997 (07.08.97), formula I	1-14
X	STN International, File CAPLUS, CAPLUS accession no. 1978:165488, Kyowa Hakko Kogyo Co., Ltd. et al: "Prevention of crop plant damage cased by N-substituted phenylcarbamoylamino acid herbicides"; & JP,A2,52154523, 19771222, formula I	1-14
X	EP 0743067 A2 (TORAY INDUSTRIES, INC.), 20 November 1996 (20.11.96), claims 1-36, formula I	1-19,21
Y	WO 02076930 A2 (TELIK, INC.), 3 October 2002 (03.10.02), claim 22	1-19,21
Y	WO 02083628 A1 (BOEHRINGER INGELHEIM PHARMACEUTICALS, INC.), 24 October 2002 (24.10.02), formula I	1-19,21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 03/01434

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Amalytica Chimica Acta, Volume 399, 1999, Petra M. Krämer et al: "Flow injection immunoaffinity analysis (FIIAA) - A screening technology for atrazine and diuron in water samples", page 89 - page 97, Diuron ---	1-19,21
A	Journal of Immunological Methods, Volume 196, 1996, G.A. Bonwick et al: "Production of murine monoclonal antibodies against sulcofuron and flucofuron by in vitro immunisation", page 163 - page 173, figures 1, 5 ---	1-19,21
A	US 2002193633 A1 (ZIXIA FENG ET AL), 19 December 2002 (19.12.02), scheme 1, claims 1-19 ---	1-19,21
A	EP 0327365 A2 (HYBRITECH INCORPORATED), 9 August 1989 (09.08.89), claims 1-32 --- --------	1-19,21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE03/01434

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 20
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet*

2. ☒ Claims Nos.: 13 and 14
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see next sheet**

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

*
Claim 20 relates to a method for isolating human IgG of K-type from other components in a liquid. In view of the large number of urea compounds found and also the lack of clarity of claim 20 regarding the steps of the method, which render it difficult, if not impossible, to determine the matter for which protection is sought, the present application fails to comply with the clarity and conciseness requirements of Article 6 PCT (see also Rule 6.1(a) PCT) to such an extent that a meaningful search on the basis of the claim is impossible.
Consequently, no search has been carried out for the claimed method in claim 20.

**
Present claims 13-14 relate to a compound defined by:
P1: Binding constant (10e-3M) or,
P2: Structure ccordinates of a binding pocket.
The use of these parameters in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to compare the parameters the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a meaningful complete search impossible. Consequently, the search has been restricted to: The parts relating to the compounds given in Formula I relating urea-like compounds.

INTERNATIONAL SEARCH REPORT

Information on patent family members

31/10/03

International application No.
PCT/SE 03/01434

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